Genetic assessment of five breeding populations of abalone (*Haliotis midae*) through a comparative Performance Testing Scheme

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Agriculture

at
Stellenbosch University
Department of Genetics, Faculty of AgriSciences

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March 2015

Declaration

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Summary

Cultured abalone in South Africa is undomesticated. For the local industry to remain competitive on the international markets it is essential to improve production. This study is part of a selective breeding component of a larger genetic programme that aims to enhance productivity of the local industry by genetic improvement of growth rates.

Selective breeding programmes are based on genetic variation and correlations. Molecular studies proved genetic differentiation exist between the broodstock- and offspring populations and among the offspring populations used in this study.

Five commercial abalone farms from the Walker bay region each entered 3000 randomly selected animals obtained from synchronised mass spawning of conditioned broodstock into a Performance Recording Scheme (PRS). Microsatellite marker analysis proved these broodstock populations to be representative of the wild populations. The five cohorts were assessed over the five locations represented by three replicates per location with 200 randomly assigned animals per replicate. The average growth rate was used as growth performance parameter by measuring shell length and body weight at three month intervals over a period of 24 months.

Interaction was observed between cohort and location effects when analysing the full data set. This was unexpected as the cohorts were constructed from parent stick that was randomly sampled from the same geographical area, the larger Walker bay. The factors suspected of causing this observed interaction were considered in a stepwise analysis. Initial and progressive tag loss, differences in initial size of animals entered into the study and on-farm management errors were considered as possible causes of the observed interaction in a stepwise analysis.

Statistically significant differences were observed between the five cohorts and between the five locations in terms of length and weight growth rates. Based on these findings it is advised that a central facility is used to effectively compare the growth rates of different cohorts or populations. Any future research in selective breeding to follow this study should involve the integration of molecular techniques and biotechnologies.

Opsomming

In Suid-Afrika is gekweekte perlemoen wild. Vir die plaaslike bedryf om op die internasionale markte kompeterend te bly, is dit noodsaaklik om produksie te verbeter. Hierdie studie vorm deel van ’n selektiewe telingskomponent van ‘n groter genetiese program met die doel om die produktiwiteit van die plaaslike bedryf deur die genetiese verbetering van groeitempo’s te verbeter.

Selektiewe teelprogramme word gebaseer op genetiese variasie en korrelasies. Molekulêre studies het bewys dat daar genetiese differensiasie bestaan tussen die teel- en nageslagpopulasies en onder die nageslagpopulasies wat in hierdie studie gebruik is.

Vyf kommersiële perlemoenplase in die Walkerbaaistreek het elk 3000 ewekansig geselekteerde diere vanaf gesinkroniseerde massa broei van gekondisioneerde teelpopulasies aan ‘n *Performance Recording Scheme* (PRS) bygedra. Mikrosatelliet merker-analise het bewys dat hierdie teelpopulasies verteenwoordigend is van die wilde populasies. Die vyf kohorte is oor die vyf liggings geassesseer, elkeen waarvan verteenwoordig is deur drie replikate bestaande uit 200 ewekansig toegedeelde diere per replikaat. Die gemiddelde groeitempo is gebruik as die groeiprestasieparameter deur skulplengte en liggaamsgewig elke drie maande oor ‘n tydperk van 24 maande te meet.

Daar is interaksie waargeneem tussen kohort- en liggingseffekte toe die volledige datastel geanaliseer is. Hierdie was onverwags, aangesien die kohorte gekonstrueer is uit teelouers waarvan monsters ewekansig vanuit dieselfde geografiese gebied, naamlik die groter Walkerbaai, geneem is. Die faktore wat vermoedelik hierdie waargenome interaksie veroorsaak het, is in ‘n stapsgewyse analise beskou. Aanvanklike en progressiewe merkerverlies, verskille in die aanvanklike grootte van die diere wat in die studie ingesluit is en bestuursfoute op die plaas is as moontlike oorsake van die waargenome interaksie voorgestel.

Statisties betekenisvolle verskille is tussen die vyf genotipes en tussen die vyf liggings in terme van lengte en gewigsgroeitempo’s waargeneem. Op grond van hierdie bevindings word daar voorgestel dat ‘n sentrale fasiliteit gebruik word om die groeitempo’s van die verskillende genotipes of populasies doeltreffend te vergelyk. Enige toekomstige navorsing oor selektiewe teelt wat op hierdie studie sou volg, moet die integrasie van molekulêre tegnieke en biotegnologieë behels.

This thesis is dedicated to my parents, Nico and Babelie Vlok,
and to the glory of God

Biographical sketch

Arnold Vlok was born 21 May, 1984. He attended Stellenbosch High School and matriculated in 2002. He enrolled for and completed a BSc-degree in Animal Biotechnology at Stellenbosch University. He worked as project coordinator on a project aimed at the genetic improvement of South African indigenous abalone, *Haliotis midae,* while completing this study.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- My parents, Nico and Babelie, and my brothers, Etienne and Eugene for their love and support
- Alet de Wet for incredible emotional support and encouragement
- My friends for their encouragement, especially Izak Saayman
- Prof Danie Brink for his guidance and input
- Gareth Difford for shedding light on statistics
- The participating abalone farms
- Mrs Annalene Sadie for making sense of data
- The Aquaculture certificate students for months of help and fun, tagging and sampling
- Karin Vergeer for all the editing

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Abbreviations and symbols

%	Percentage
<	Less than
(Pty) Ltd	Property limited
ABA	Abagold Ltd
ADLG	Averge Daily Length Gain
ADWG	Average Daily Weight Gain
ANOVA	Analysis of variance
Aqua	Aquafarm Development (Pty) Ltd
BLUP	Best Linear Unbiased Prediction
CV	Coefficient of variation
d	Day
FAO	Food Agriculture Organisation of the United Nations
g	Grams
GLM	General Linear Models
h	Hours
H_a	Alternative Hypothesis
H_o	Null Hypothesis
HIK	HIK Abalone Farm (Pty) Ltd
I&J	Irvin & Johnson Ltd
L	Length
LS-Means	Least-Square Means
mm	Millimetres
N	Count
p	Statistical probability
Std Dev	Standard deviation
r	Correlation coefficient
RB	Roman Bay Sea Farm (Pty) Ltd
TM	Trademark

Chapter 1

Introduction

Chapter One: Introduction

1.1 South African Marine Aquaculture

Aquaculture is defined as the farming of aquatic organisms including fish, molluscs, crustaceans and plants in controlled or selected aquatic environments, with some form of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators (Department of Environmental Affairs and Tourism, 2012)

The 3000 km coastline of South Africa stretches from the Orange River on the west coast to Ponta do Ouro on the east coast. South Africa is unique in having contrasting currents on opposite coasts as well as the Indian and Atlantic oceans (Harrison, 2002; Department of Environmental affairs, 2012). The colder Benguela Current flows up the west coast and the warmer Agulhas Current flows down the east coast (Shipton and Britz, 2007; Department of Environmental Affairs, 2012). The South African coast also spans three bio-geographical zones. The cool temperate west coast, the warm temperate south coast and the subtropical east coast (Figure 1.1) (Department of Environmental Affairs, 2012). These diverse climatic conditions provide the potential to cultivate a wide variety of marine species.

Aquatic food products are derived from capture fisheries and aquaculture production. Some of the main fishing areas have reached sustainable level with global production levelling off. The production from capture fisheries is considered unable to sustain the growing demand for aquatic food (Hecht *et al.,* 2006). Population growth, rising per capita income and urbanisation are expected to fuel the demand for marine products, which can only be met by expansion of aquaculture production.

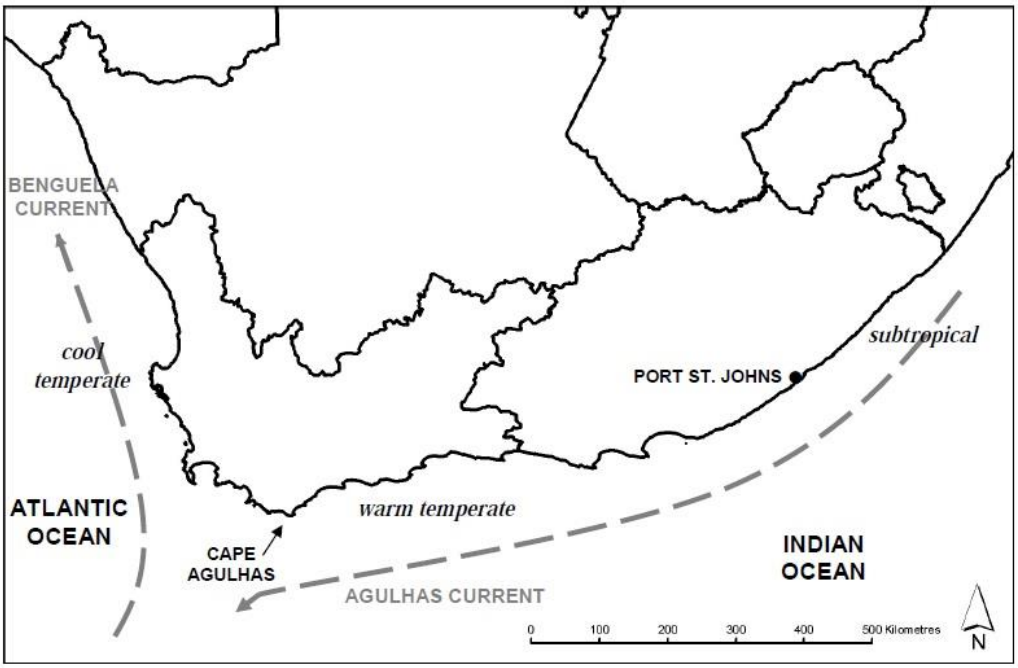

Figure 1.1 Map of South Africa showing the bio-geographical zones and the two major currents. The cool Benguela Current and the warmer Agulhas Current. (Van der Merwe, 2009)

Commercial aquaculture production has taken off recently in response to the sharp rise in fish prices according to an FAO survey in 2006 (Hecht *et al.,* 2006; Brugère, 2004). Excluding plants the global aquaculture output accounted for 40.1% of total fisheries production in 2011 compared to only 3.9% in 1970 (FAO, 2015)

The African aquaculture sector has been slow to respond to the rising demand for aquatic products, however South Africa already has a well-established value chain for aquatic products due to the modern infrastructure that supports the harvest fisheries. During the 1990's local aquaculture producers targeted the international market due to a favourable weak local currency and elevated prices abroad. Economic conditions in recent years including a more volatile rand, increased energy costs and the lowering of production cost in China and other East Asian countries negatively affected producers who rely on the export market. South African abalone, *Haliotis midae* is the exception in that it is an established premium product in Asian markets which, is linked to a strong economy and per capita income in East Asian countries (Hecht *et al.,* 2006).

Aquaculture species cultured in South Africa include high value mollusc species such as abalone, mussels and oysters; and finfish such as dusky kob (*Argyrosomus japonicas*), silver kob (*Argyrosomus inodorus*) and yellowtail (*Seriola lalandi*). Seaweed is produced exclusively as feed for abalone. By the end of 2011 there were 30 operating aquaculture farms in South Africa with

the Western Cape Province comprising 20 of them (Table 1.1) (Department of Agriculture, Forestry and Fisheries, 2012a).

Table 1.1 Total number of aquaculture farms operating in South Africa by province and sub-sector in 2011 (Department of Agriculture, Forestry and Fisheries, 2012a)

Number of farms cultivating species in each province					
Species	Western Cape	Eastern Cape	Northern Cape	Kwazulu-Natal	Total
Abalone	11	1	2	0	14
Finfish	0	2	0	1	3
Mussels	3	0	0	0	3
Oysters	6	3	1	0	10
Total	**20**	**6**	**3**	**1**	**30**

The abalone sub-sector is the highest contributing sub-sector in terms of production comprising 55 percent of total production. The high value of the product led to abalone production contributing 93 percent of the total value of aquaculture production (Figures 1.2 and 1.3) (FAO, 2015)

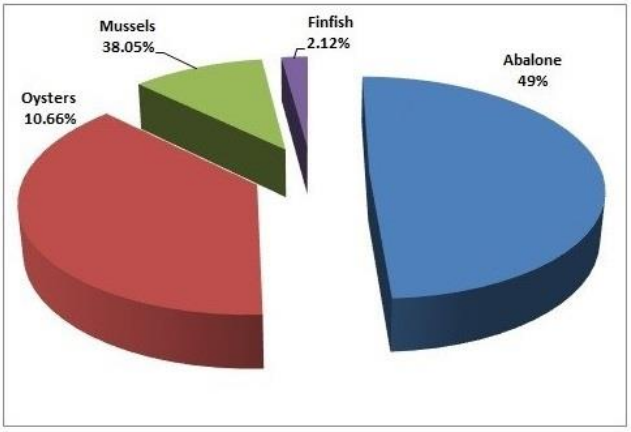

Figure 1.2 Percentage contribution of each sub-sector to total production in 2012 (FAO, 2015)

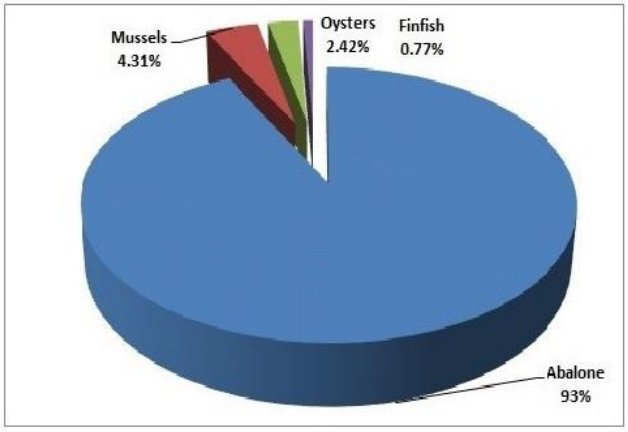

Figure 1.3 Estimated percentage contribution of each sub-sector to total value of production (FAO, 2015)

The marine aquaculture industry provides employment, socio-economic development and food security through income to coastal areas. With the anticipated development of services, such as governance, security, packaging, feeds, processing, transport and research, it is expected that production and employment figures could be doubled in a 10-15 year period (Shipton and Britz, 2007; Department of Agriculture, Forestry and Fisheries, 2012b).

1.2 Abalone Fisheries and Farming

Abalone species in the genus *Haliotis* are commercially valuable and sought after because of the large edible abductor muscle and foot (Arai and Okumura, 2013). The international trade in abalone is driven by exceptional demand and high prices in Asia (Raemaekers *et al.,* 2011). This has led to the farming of 12 different *Haliotis* species in 16 countries (Franchini *et al.*, 2011b).

Abalone are easily exploitable in the wild due to their inactive nature. The increase in abalone prices and the difficult political landscape in South Africa in the 1990's triggered a rise in illegal trade of abalone which ultimately led to the closure of the quota-managed fishery in 2008. The fishery was later reopened in July 2010 based on total allowable catch (TAC) allocations conditional to reduction in poaching (Department of Agriculture, Forestry and Fisheries, 2012b).

Only *H. midae* of the six *Haliotis* species that occur in South Africa is commercially exploited (Hauck and Sweijd, 1999). Attempts to cultivate *H. midae* first occurred in 1981 although the South African abalone fishery has existed since 1949 (Sales, 2001). From a measly production of approximately one ton 1993 abalone production in South Africa steadily increased to reach 1036 tonnes in 2011 with a value of 40.87 million US Dollars (Figures 1.4 and 1.5) (FAO, 2015). South Africa is already the largest producer of farmed abalone outside of Asia (Bolton *et al., 2009*) and with the current focus on market growth and improved technology there is further scope for the industry to expand.

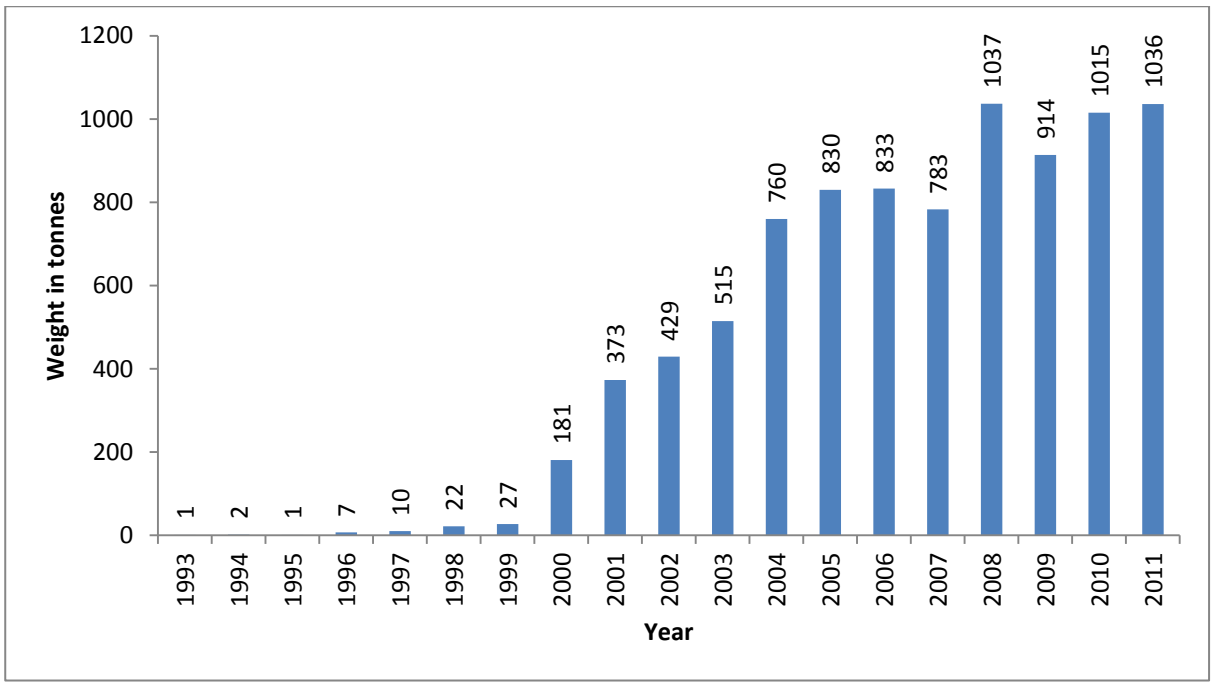

Figure 1.4 Weight in tonnes per year of farmed abalone in South Africa (Graph constructed using data from the FAO, 2015)

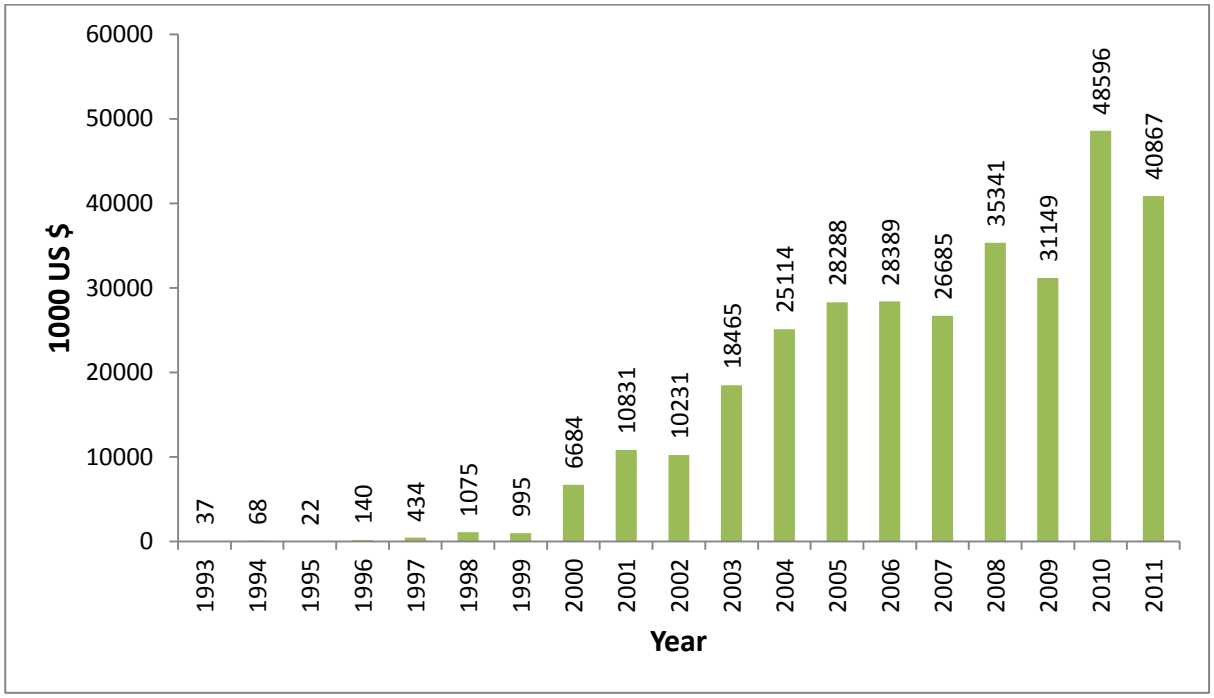

Figure 1.5 Value in US $ per year of farmed abalone in South Africa (Graph constructed using data from the FAO, 2015)

Abalone farms in South Africa are situated all along the coast from Port Nolloth on the west coast to East London on the east coast. The majority of farms are located in the southern coastal area with seven farms in the Hermanus and Gans Bay area known as Walker Bay; and three farms near Saldanha Bay (Department of Agriculture, Forestry and Fisheries, 2012).

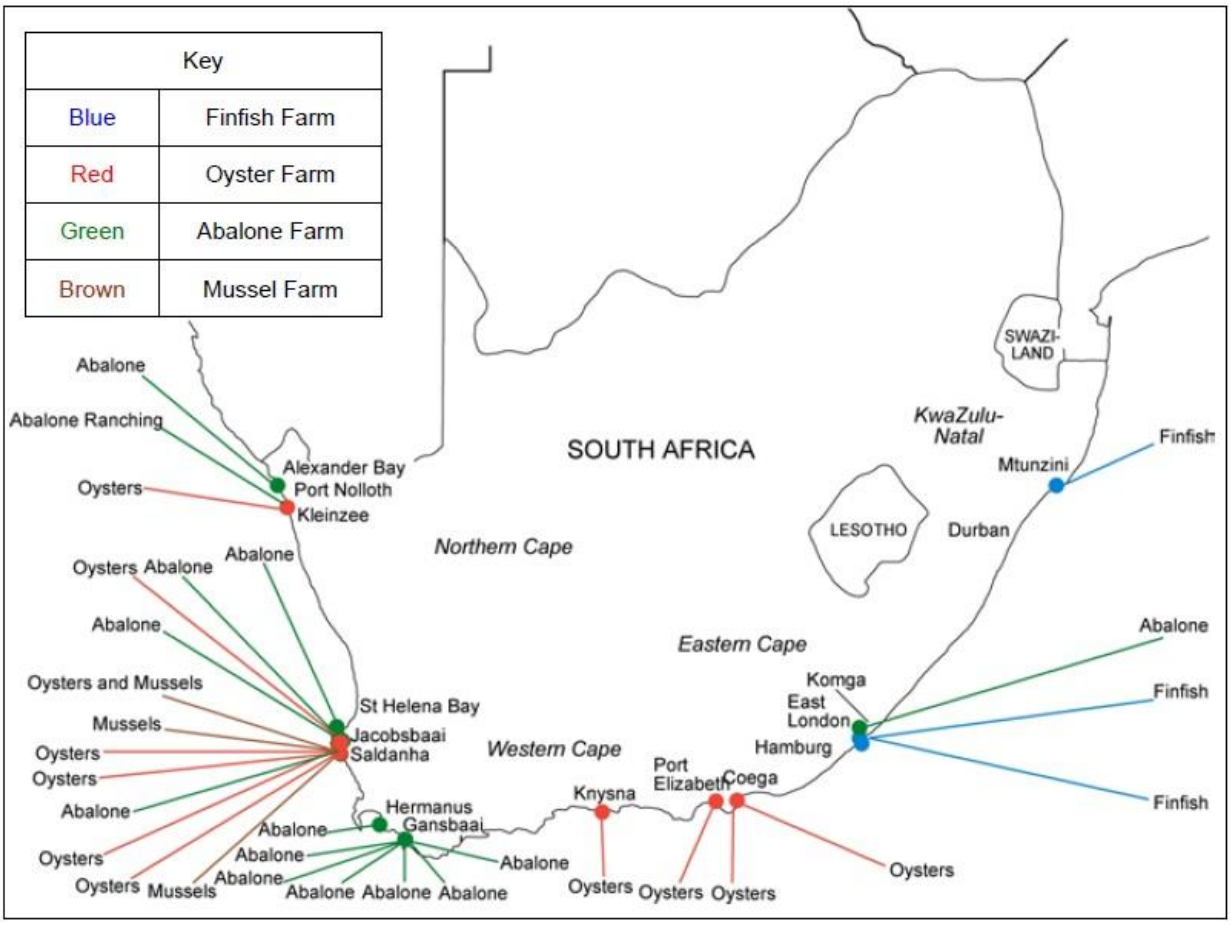

Figure 1.6 Map of South Africa showing location of aquaculture farms in 2011 (Department of Agriculture, Forestry and Fisheries, 2012a)

Abalone culture in South Africa is based on shore-based pump-ashore systems (Shipton and Britz, 2007; Sales and Britz, 2001). The abalone are reared on seaweeds and artificial diets and stocked in high densities (Sales, 2001).

Haliotis midae does not readily spawn in captivity if collected ripe from the wild unlike other abalone species though technology to spawn *H. midae* artificially has subsequently been established (Sales and Britz, 2001). After spawning and fertilisation, the metamorphosed abalone are reared on diatoms either on plastic plates or in plastic bags. They are eventually weaned on to seaweed or formulated feed after reaching a shell length of 4-6 mm (Sales, 2001). With a growth rate of 2-3 cm per year it takes an abalone three to four years to reach a market size of 85 mm to 100 mm (Franchini *et al.*, 2011a; Shipton and Britz, 2007).

Haliotis midae enjoys consumer preference on global markets due to its appearance and taste and is one of the most valuable commercial abalone species and a highly valued marine product (Sales and Britz, 2001; Van der Merwe, 2009). The prime market is Asia where abalone products are used

in traditional cuisine and ceremonies (Sales, 2001). The shells are used as jewellery and decoration. The quality of the meat depends on the size, texture and colour. The size preference of the market can be met by varying the time of harvest (Oakes and Ponte, 1996). The meat of *H .midae* is light in complexion and lacks pigment which makes it aesthetically desirable and demands a higher price in comparison to most other species.

1.3 Project Aims

The commercial broodstock of cultured abalone in South Africa are obtained from natural populations and considered as undomesticated. The broodstock are representative of wild populations they are sourced from and have not adapted to husbandry conditions. Defined genotypes or strains have not been developed for cultivation. A genetic improvement program was established in 2006 by a consortium of producers in the South African abalone industry together with the University of Stellenbosch.

The programme aims to incorporate selective breeding, molecular genetics and biotechnology strategies to genetically enhance growth rate as the main economically important trait, to ensure that the South African industry remain globally competitive. Conventional selective breeding is based on controlled mating and can only improve the desired trait when genetic variance occurs between individuals in the population (Falconer and Mackay, 1996).

This study aims to accurately assess the production performance and the variance among the five participating hatcheries and geographical locations through the establishment of a performance recording scheme (PRS). Shell length and live weight measurements are used to assess the regression coefficients of average length and weight gain as indicators of growth rate. The objectives of the study were to assess whether any statistically significant differences occur between the offspring of the five participating hatcheries (cohorts) and between the five geographical locations in terms of average length and weight gain. It was also necessary to investigate whether there was any significant interaction between the main effects.

1.4 Summary

Cultured abalone in South Africa is undomesticated. This study is part of the selective breeding component of a larger genetic programme that aims to enhance productivity of the local industry

by genetically improving the growth rate. Knowledge of the variance among individuals and populations is essential in establishing a selective breeding programme.

It is essential for the local industry to improve production to remain competitive on the international market.

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Chapter 2

Literature Review

Chapter Two: Literature Review

2.1 Biology of South African Indigenous Abalone, *Haliotis Midae.*

2.1.1 Taxonomic classification

Abalone are univalve marine gastropods that belong to the genus *Haliotis* (Elliot, 2000). *Haliotis* is part of the phylum, Mollusca, which is the largest phylum in marine waters and includes 23% of marine animals (Van der Merwe, 2010). Mollusca is the second largest phylum in the animal kingdom and species include chitons, snails and abalone, oysters and octopuses. Taxonomic classification is presented in Tables 2.1 below.

Table 2.1 Taxonomic classification of *Haliotis* (Van der Merwe, 2010; Elliot, 2000)

Phylum	Mollusca
Class	Gastropoda
Subclass	Orthogastropoda
Superorder	Vetigastropoda
Family	Haliotidae
Genus	*Haliotis*

Haliotis belong to the order Vetigastropoda under the class Gastropoda. There are six haliotid species indigenous to Southern Africa as summarised in Table 2.2.

Table 2.2 *Haliotis* species and its occurrence in Southern Africa (Schoonbee, 2008)

Species	**Distribution**
H. midae	Saldanha to Port St. Johns
H. parva	Cape Town to East London
H. spadicea	Cape Town to Sodwana
H. alfredensis	Port Alfred to Port St. Johns
H. queketti	East London to Durban
H. pustulata	North of Sodwana

Haliotis midae is the largest of the South African species and the only one commercially exploited (Hauck and Sweijd, 1999).

2.1.2 Biology and anatomy

An oval shaped shell covers most of the abalone. The shell is in the form of a spiral that spirals outwards from the posterior apex toward the anterior side (Fallu, 1991; Fishtech^{TM}Inc, 2014). Respiratory pores occur along the outer edge of the shell increasing in size toward the anterior side. The posterior, older pores close up as growth proceeds. Abalone are permanently attached to their shell through the muscle attachment. The shell is formed during the larval stage and abalone are reliant on it for protection throughout its lifespan for protection against predation and other environmental factors (Fishtech^{TM}Inc, 2014).

Figure 2.1 Dorsal view of the abalone (Photograph, Gert le Roux; Van der Merwe, 2009)

The large muscular foot and the anterior head is located underneath the shell. The abalone can clamp down tightly on substrate through strong suction of the muscular foot (Sales and Britz, 2001). The foot is encircled by the mantle and sensory extension of the foot called the epipodium. The epipodium bears tentacles that can project beyond the edge of the shell (Fishtech^{TM}Inc [online], 2014).

The abalone's internal organs are hidden between the shell and the foot and include a pair of eyes, a mouth with an elongated tongue or radula, two enlarged tentacles and a gonad (De Beer, 2008; Fallu, 1991). The gill chamber is located next to the mouth. Water is drawn through the

respiratory pores, over the gills and out the pores again carrying reproductive products and metabolic waste (Fallu, 1991). The abalone feeds by a rasping action of the tongue-like, abrasive radula. The radula has a toothed surface which is pressed against the algal food source which scrapes off fine particles (Purchon, 1977).

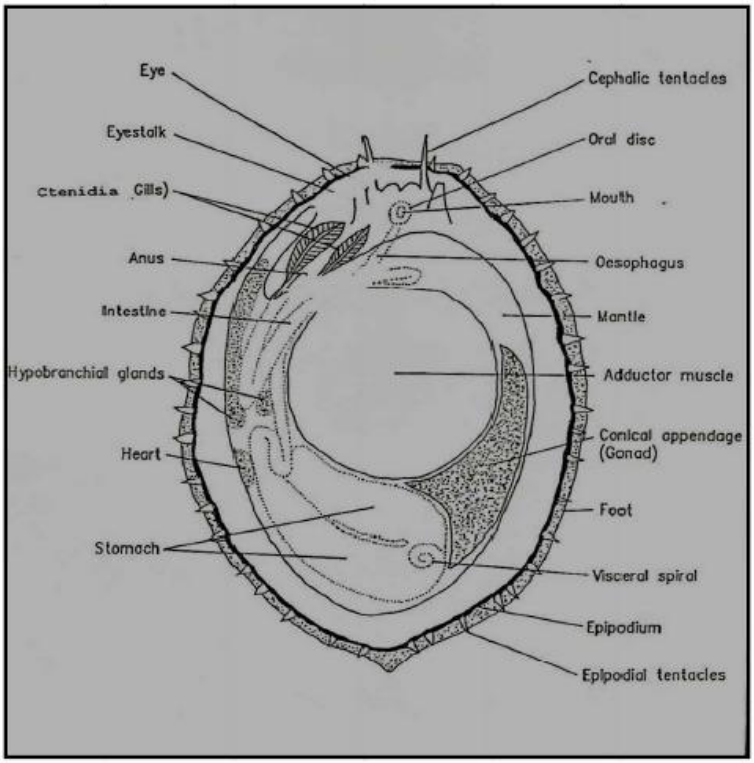

Figure 2.2 Ventral view of organs and soft body parts of the abalone (Van der Merwe, 2010)

The gonad envelops the ducts of the gut and is visible when lifting the epipodium away from the shell (Fallu, 1991). The eggs of females are green and sperm of males are beige.

Abalone has a clear haemolymph that transport oxygen and carbon dioxide through the gills and body. The haemolymph is pumped by muscular contractions of the heart and contains no clotting agents. Any cut or abrasion will almost certainly lead to the abalone bleeding to death (Anderson, 2003). The abalone possesses two kidneys with different functions. When haemolymph pressure is greater than the colloidal osmotic pressure of the haemolymph, ultra-filtration is possible through the arterial walls. After filtration trough the arterial walls, the filtrate is processed through secretion of the right kidney and reabsorption of the left kidney (Vosloo and Vosloo, 2006).

2.1.3 Reproduction and life cycle

Studies found that South African abalone, *H. midae,* reaches 100% sexual maturity at the age of around 7.2 years. Under controlled conditions and on the warmer east coast sexual maturity can occur as early as three years of age (Tarr, 1995). Spawning occurs during spring and autumn depending on the locality. Two discrete groups of eggs are found within a ripe ovary that are released on consecutive spawning (Sales and Britz, 2010). Abalones reproduce through broadcast spawning. Sperm and eggs are released through a small duct next to the anus into the surrounding water through pores (Anderson, 2003).

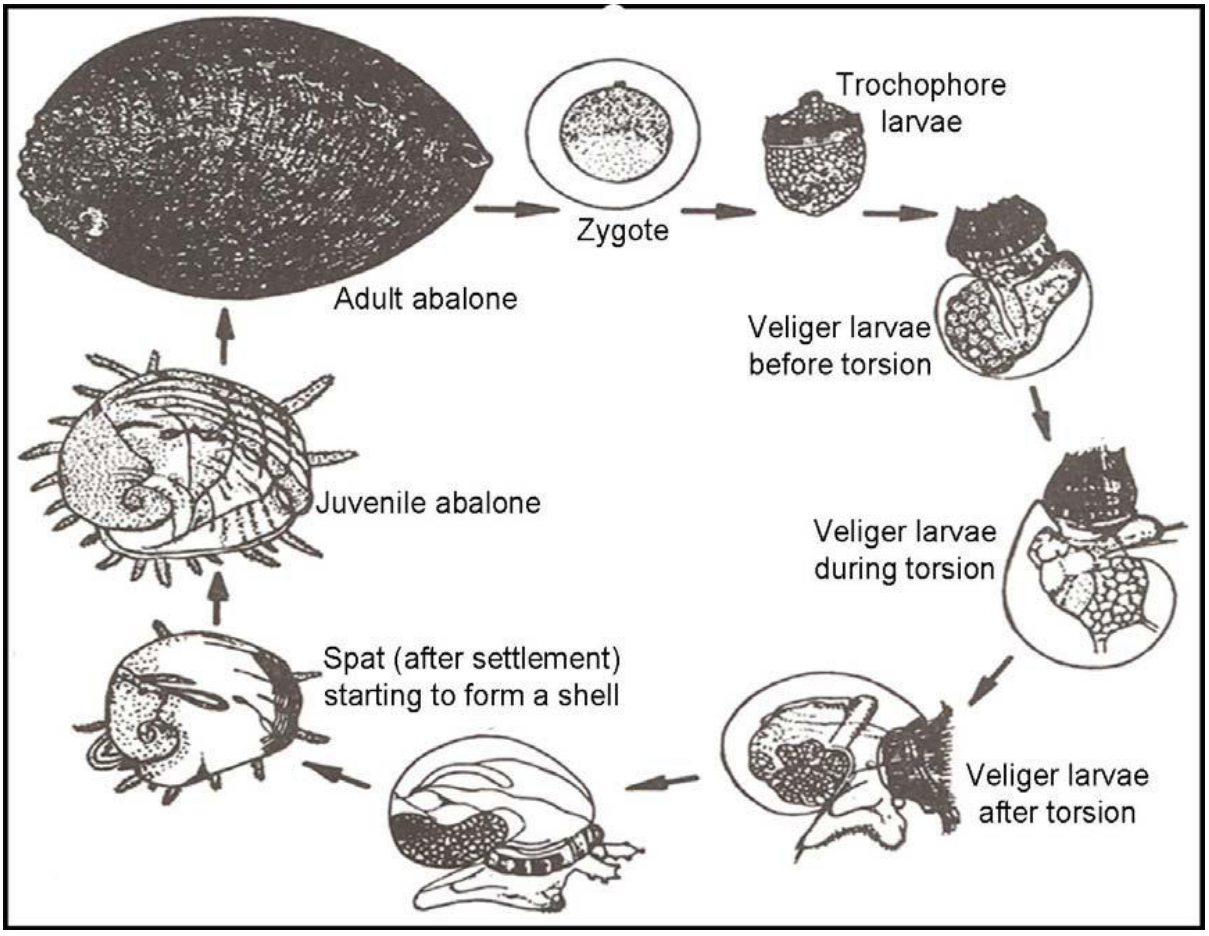

Figure 2.3 Illustration of the abalone life cycle (Rhode, 2010)

After fertilisation the eggs are approximately 0.2mm in diameter and undergo a series of divisions to reach the trocophore stage where they are classified as free-swimming larvae. The trocophores then develop further to become photophobic and settle into obscure habitats. At this stage they are classified as juveniles or “spat” (Ruivo, 2007). The larvae settle down in shallow water, hiding under substrate and develop into abalone (Sales and Britz, 2007).

2.1.4 Growth and feeding

Within their natural environment abalone are slow feeding, nocturnal herbivores (Van den Berg, 2008). Juveniles feed on micro-algae and diatoms attached to the surfaces onto which the abalone settles. Adults feed mainly on seaweeds (Elliot, 2000). In production systems the abalone are fed formulated foods or macro algae including kelp (*Ecklonia maxima*), cultured *Gracilaria spp* (e.g. *Gracilaria verrucosa*), or combinations of these (Sales and Brits, 2001).

In the wild abalone can reach a maximum size of 200 mm after 30 years of growth. Farm production is concentrated on the abalone reaching a marketable size of 100 mm after five years. Under stimulated farming conditions on formulated diets, growth rates of 0.08 to 4.5 % body weight per day of abalone of 10-17 mm shell length has been reported (Sales and Britz, 2001). The corresponding feed conversion ratio (FCR) was 0.9 to 2.4. Optimal growth rate and FCR is achieved between 12 ^{o}C to 20 ^{o}C (Sales and Britz, 2001). Several factors within the husbandry system affect the growth rate and FCR of abalone. These include ambient water temperature, daylight length, water quality, feed quality and stocking density (Vorster, 2003). Valuable improvements in growth can be achieved through breeding techniques and genetic research (Huang and Hseu, 2010).

2.2 Physiological aspects related to growth measurement

2.2.1 The shell

The *Haliotis* larvae form a primitive shell called the protoconch through secretion by the shell glands located in the embryo. The shell is spiral shaped and protects the larvae. After the embryonic stage the shell develops to consist of three layers: The outer periostracum, the prismatic layer composed of calcite crystals and the inner nacre composed of aragonite (Bevelander, 1988).

The protoconch forms the apex of the shell. The growth of the shell is achieved through the deposition of the new shell material by the mantle on aperture of the shell. An abalone shell never stops growing. It increases in length up to about 200 mm after which only thickening occurs (Schoonbee, 2008). In abalone aquaculture the stocking density of the abalone in baskets affects

the competition for food and shelter. This leads to breakages in the shell and a reduction in formation of the shell (Huchette *et al.*, 2003). The nature of the shell growth dictates that juvenile abalone reflect the breakages and other factors affecting growth in their shells. The shell quality of a batch of animals indicates the health and conditions of that batch (Schoonbee, 2008).

2.2.2 Tagging

An effective tagging method must be non-invasive to the animal and durable for long term identification. The tagging of large numbers of small abalone is especially difficult. Larger animal are traditionally tagged by adhering a tag to the shell with an adhesive such as Pratley Putty or by attaching a tag to the larger shell pores (Schoonbee, 2008). The smaller abalone (5-10 mm shell length) has smooth and fragile shells without any distinguishable ridges and very small pores. Two tagging methods are used to tag the smaller abalone. Elastic silicone tags are inserted and lodged in the breathing pore or a small tag is glued onto the smooth shell with a liquid quick-set adhesive (Superglue). The silicone tag causes irritation of the foot muscle of the abalone when it is not lodged perfectly, a feat which is hard to achieve. It also displayed severe tag loss within the first six months. The Superglue method proved non-evasive and effective although some tag loss was experienced. Aerial exposure to external elements negatively affects the growth of juvenile abalone due to stress.

2.2.3 The foot

The foot of the abalone is used for movement, feeding and attachment to surfaces. Several factors influence the growth of the foot of juvenile abalone. These include water quality variable like dissolved oxygen, depth, water flow, temperature, salinity, food quantity and quality, stocking density, and the husbandry system (Huchette *et al.*, 2003).

2.3 Life stages of *Haliotis midae* relevant to the study

2.3.1 Larval development

Abalone eggs hatch as free-living larvae that drift for approximately seven days during which 41 stages of larval development can be identified before metamorphosis. Two distinct phases can be identified: 1) trochocphore larval stage, and 2) the veliger larval stage. Abalone are ectotoerms and are dependent on water temperature during larval development. Depending on the water temperature it takes on average about 20 h for the trochophores to develop into veliger larvae. Veligers develop a head and a foot and sink to attach themselves to a substrate to undergo the final stage of metamorphosis and begin to develop a shell (Takami *et al.*, 2001).

Table 2.3 The stages of larval development of *H. midae* at 20^{o}C (Schoonbee, 2007)

Stage	Description	Time from fertilization	
		Hours	~Days
1	Hatching	14	
2	Free-swimming trocophore	22	
3	Cap-shell, early veliger	24	1
4	Inflate-shell veliger	31	
5	Early operculate veliger, pre-eyespots	46	2
6	Incipient cephalic tentacle, operculate veliger	51	
7	Mid-formed cephalic tentacle	86	3
8	Digitate (branched) cephalic tentacle	97	4
9	Crawling, settlement	118	5
10	Total metamorphosis	145	6
11	Peristiomial growth	169	7

The survival rate of larvae to adulthood in the wild is very low as mortality rates are above 99% (Schoonbee, 2008).

2.3.2 Settlement

Settlement is the most critical stage of abalone development and occurs a week to a month after the veliger stage depending on the conditions. This is when the larvae reach the bottom and start crawling and looking for substratum to attach to (De Beer, 2004). At this stage they are called “spat” and start feeding on micro-algae (Fallu, 1991). Both the chemical and physical

characteristics of the substrate play a role in the rate of settlement. Settlement rates are highly variable and unpredictable due to the variable conditions in water temperature, substrate composition and the available feed (Schoonbee, 2008).

2.3.3 Growth and feeding

Wild *H. midae* feed intake is estimated at 8.1% of wet body weight per day at 14^{o}C and 11.4% at 19^{o}C (Sales and Britz, 2001). Barkai and Griffiths (1988) found that the absorption efficiency on a natural diet is estimated at 37.25%. Of the energy derived from food 63% is excreted as faces and 32% used for respiration, which leaves only 5% available for growth and reproduction. Abalone are sensitive to external and environmental conditions. Stress brought on by external stimuli can cause energy to be used in stress response which in turn affects feed intake and growth (Huchette *et al*., 2003).

Stocking densities in holding units influence the competition amongst abalone for available feed. The availability of feed is affected by the amount of feed per abalone and the ability of the abalone to reach it (Schoonbee, 2008).

2.3.4 Movement

Juvenile abalone are photosensitive and will move away from sunlight. They forage during the night and will move to where food is available. When the holding unit does not provide sufficient protection form sunlight or the competition for food is too high, the abalone will leave the unit in search of food and covered areas (Huchette *et al.,* 2003). These animals often die due to exposure (Schoonbee, 2008).

2.4 Genetic improvement strategies

Unlike a variety of farm breeds of terrestrial animals, very little development has occurred of domesticated and high-performing farm breeds in aquaculture. According to Bentsen and Olesen (2002) aquaculture production will have to increase to 63 million tonnes by 2025 to meet expected demands. The development of domesticated and genetically improved aquaculture breeds is crucial to achieve this feat (Bentsen and Olesen, 2002). Abalone is an attractive

aquaculture species since it has an established market, which demands a high price on international markets (Oakes and Ponte, 1996). Slow growth in abalone species is the most serious problem concerning its market competitiveness (Arai and Okomura, 2012). Genetic improvement programmes are needed to ensure the competitiveness of local abalone as international cultured production increases. Elliot (2000) reported production gains of between 5% and 15% per generation for species other than abalone through structured genetic breeding programmes.

Elliot (2000) defines genetic improvement as the gain in the cultured production of the abalone species through the exploitation or manipulation of the genetic variation present within the particular species. In any culturing system there are generally four inputs that affect production gain – management practices, nutrition, farm size and genetics. The biological potential of the species to exploit its environment is determined by its genetics. The other inputs are environmental and are mainly targeted by farm managers to increase production (Elliot, 2000).

Genetic improvement programmes in aquaculture are normally aimed at producing faster growing animals as it is intrinsically linked to profitability and productivity (Franchini *et al.*, 2011a). As in most aquaculture species, the focus with *H. midae* is the improvement of growth rate. Genetic improvement strategies can also be used to improve other traits such as feed conversion efficiency, disease resistance, flesh quality, better meat yield, higher fecundity, sterility and enhancement of pearl production (Franchini *et al.*, 2011b).

Genetic variation and correlation between genotype, phenotype and environment are at the core of the quantitative evolutionary theory. High individual variation is normally found in growth rates of wild animals. This is the material on which selection strategies are built (Falconer and Mackay, 1996). By acquiring larger samples of relatable individuals more accurate estimations can be made of parameters such as heritability of traits and genetic correlations (Sales and Britz, 2001). There are two types of genetic variation. Genotypic variation refers to the genetic make-up of an individual. This area is targeted by molecular genetics techniques. Phenotypic variation is the physical expression of the genotype. These traits can be measured or described (Elliot, 2000).

2.4.1 Genetic markers

Genetic markers have several uses in genetic breeding programmes, including the quantification of genetic variation, inbreeding and breeding numbers, assisting in pedigree analysis and locating genes (Elliot, 2000).

Studies in livestock indicate that genetic improvement can be accelerated even further by using marker assisted selection (MAS). Especially if markers surrounding quantitative growth loci affecting the desired trait are available (Hayes *et al.*, 2007). Quantitative trait loci (QTL) are defined by Seaton *et al.* (2002) as stretches of DNA containing or linked to the genes that underlie a quantitative trait. The presence of the QTL indicates that the desired phenotype is present in the individual. The efficiency of MAS coupled with traditional selective breeding is influenced by factors such as the selection scheme used and the heritability of the desired trait. Sonesson (2007) found that MAS had up to twice the genetic gain of a corresponding scheme that did not use MAS. The relative efficiency for MAS increases with higher sample size and lower heritability of the trait. Genetic marker maps are available for a few aquaculture species normally made up of amplified fragment length polymorphism markers that are anchored to microsatellites. Relatively few QTL for desired traits are available in aquaculture compared to domesticated animal species (Sonesson, 2007). The productivity of farmed abalone can be greatly improved through the knowledge of QTL and traits of interest and the implementation of MAS (Franchini *et al.,* 2011b). Another advantage of MAS over traditional selective breeding is improved control of inbreeding and the potential to improve other desirable traits (Hayes *et al.*, 2007). Most breeding programmes rely on pedigree information to avoid inbreeding. These pedigrees do not exist in abalone. There is a concerted effort to increase the number of microsatellite tests in abalone. These tests can be used as markers of chromosome sections or genes to select more accurately for certain traits. These markers can also be used to infer the proportion of identical chromosome segments among abalone to avoid inbreeding (Hayes *et al.,* 2003).

Hayes *et al.* (2007) used a computer simulation model to optimise breeding strategies for marker assisted selection with best linear unbiased prediction (MBULP) for quantitative traits for southern Australian abalone. The model used five marker loci and made some assumptions including that the five loci is linked to growth rate affecting genes accounting for 50% of genetic variance in the trait, the generation interval might be reduced through improved growth rate and accounted for selective genotyping. The simulation also accounted for economic costs of running this breeding

programme and was calculated using estimates of farm running costs and expenses and costs associated with marker testing. They found that MAS provided genetic gains in the region of 15%. By using MBULP for growth the generation interval could be halved. This provided the selective breeding advantages of two generations through a single selection event.

Hayes *et al.* (2003) used a computer simulation to simulate two strategies. The first being the effect of MAS on growth rate when a DNA marker is closely linked to a genetic mutation affecting growth rate, and the second being the analysis of the rate of inbreeding when using a neutral marker not linked to a specific trait. They found a 16% increase in growth rate in the first generation of selection. By using the marker information the estimated that the rate of inbreeding can be reduced by 30%. It is clear through these studies that the use of genetic markers in selective breeding programmes can greatly improve economically important traits and monitor population parameters.

2.4.2 Conventional selective breeding

Selective breeding programmes are rarely applied in aquaculture production despite its success in other areas of primary production (Elliot, 2000). Selection is the best primitive tool for long term genetic improvement in animals, as gains are cumulative. Other approaches such as sex manipulation, hybridisation, triploidy and other molecular technologies are once off improvements and thus are reliant on starting with the strongest, selectively bred populations to achieve the best output (Bentsen and Olesen, 2002). The optimal selective breeding strategy can only be identified with the knowledge of the genetic variation in a population (Falconer and Mackay, 1996). Very little information about the genetic parameters of cultured abalone exists in literature (Kube *et al.*, 2007).

The high phenotypic variance and fecundity of abalone allows for rapid genetic improvement through high levels of selection intensity. The selection intensity is determined by the degree by which the individuals deviate from the mean, and the proportion of individuals that can be selected from individual experimental groups (Li, 2008). Almost all breeding programmes in aquaculture are aimed at improved growth rate only. It is expected that the gain in individual traits in these selective breeding programmes will reduce as the range of selected traits is increased (Gjedrem *et al.*, 2012b).

Kube *et al.* (2007) conducted a selective breeding programme of Australian Greenlip abalone (*Haliotis laevigata*). They produced 21 families from 14 parents and took length and weight measurements at four periods during the grow-out stage of the abalone's production cycle. They found economically important gains in length and weight gain even in a small population of only 21 families. A larger selective breeding programme by Symonds *et al.* (2009) established 66 families of the New Zealand indigenous abalone, *Haliotis iris*, for genetic evaluation. They also found meaningful variances in growth characteristics and an increase of variance with time. The phenotypic standard deviation (SD) of length increased from 3.45 at the age of 460 days to 5.10 at the age of 660 days while the phenotypic SD of weight increased from 0.52 to 3.34 at the same age intervals. Both reported low phenotypic variation during initial life stages. It is suspected that the genetic variation of the growth characteristics (weight and length) was masked by maternal, larval and settlement effects (Kube *et al.*, 2007). There is a very strong positive correlation between weight, length and growth rate and it was concluded that these traits are effectively genetically equivalent (Symonds *et al.*, 2009).

Li (2008) established a selective breeding programme of Australian indigenous abalone species, blacklip abalone (*Haliotis rubra*), greenlip abalone (*Haliotia laevigata*) and their hybrids. The aim of the study was to establish breeding protocol on participating farms, to investigate the feasibility of using farm facilities for the selective breeding programme and to determine the parameters of economically important traits and their correlations. In total, 235 families was established by eight participating farm hatcheries from the summer 2001/2002 to the summer of 2005/2006. The families were reared at a central facility. The study found significant genetic variation in shell length- and body weight gain, as well as positive genetic correlations between the growth traits and processing traits. This suggests that selective breeding can lead to genetic improvement in these species.

Simulation models that account for both the genetic and economic response of a selective breeding programme (bioeconomic models) provide a cost effective way of to predict its outcome (Robinson *et al.*, 2010).

Robinson *et al.* (2010) used bioeconomic models to predict the response of a breeding programme aimed at improving disease resistance and growth rate in blacklip- (*Haliotis rubra*) and greenlip abalone (*Haliotis laevigata*). They reported that if growth rate is the only selection criterion, the

greatest economic benefit to the industry would be achieved when using 150 families resulting in 12-13% improvement in initial generations. The simulation estimated a benefit/cost ration of 48/1. Li (2008) reported that improved growth rate can lead to an increase of economic benefit of >50% should a reduction in generation interval from 3 to 2 years be achieved. When using disease resistance as selection criterion the best model was to use 100 families. The constraints of this model are the risk of inbreeding and the economic costs. In theory the genetic response of a selective breeding programme is greater with larger numbers of families. The predicted genetic response was based on the heritabilities of the simulated traits estimated by Kube *et al.* (2007). The study concluded that the improvement diminishes with an increase of families, reporting similar genetic responses for simulations of 150, 200 and 250 families. Gunnes and Gjedrem (1978) define a strain as "a discrete breeding population from a river, river system, or a fjord leading to a river". Therefore any discrete breeding population from a hatchery may also be termed a strain (Ponzoni, *et al.,* 2013). The cohorts used in this study can therefore be considered as a strain. In the early stages of a breeding programme it is important to establish whether there is any strain (cohort) by environment (location) interactions between the sampled cohort and the production environment or location. If there is little or no interaction it is possible to produce a single improved strain or cohort to utilise in all production environments or locations. However if there is significant interaction, it is necessary to produce different strains for each location. In a study by Gunnes and Gjedrem (2012) on selection experiments with Atlantic salmon (*Salmo salar*) they found that the interaction between the strain and the farm environment contributed only 1-4% to the total variance. A single strain of salmon was therefore developed for all the participating farms. Eknath *et al.* (1991) tested eight strains of Nile tilapia (*Oreochromis* niloticus) over 11 production locations and found the strain by environment interaction to contribute only ~1% to the total variation in body weight. Similar results were found in a breeding programme for shrimp (*Penaeus vannamei*) by Fjalestad *et al.* (1997) where the genetic variation in harvest weight and resistance to Taura syndrome virus was measured. They concluded the interaction between the strains and the environment was insignificant.

Gjerde *et al.* (2003) studied the genotype by production system interaction for Rohu carp in monoculture and polyculture farms. They found great variation between the harvest weights of monoculture and polyculture populations and concluded that different strains had to be selected for different farms.

The economic benefits to an industry is negatively influenced by an increased interaction between strains and environments (Gjedrem, 2012a). There is no general conclusion for the level interaction within aquaculture species as illustrated by the inconsistent results of the studies mentioned. It was therefore vitally important to investigate the interaction between the cohorts and locations in this study and to determine the factors contributing to it.

Any selective breeding programme needs to make significant genetic improvement and limit inbreeding while being profitable and creating benefits for the stakeholders. It should also be commercially viable to ensure continuation of the programme. Most effects of selective breeding are not instantaneously visible and some traits are hard to quantify. It is essential to balance increased production and product quality with the costs of obtaining it to ensure sustainability in a competitive market.

2.4.3 Biotechnology

When the variation of a desirable trait is low, selection within species may be inefficient (Elliot, 2000). Inbred progeny are produced when related individuals have offspring. These progeny are almost always less fit than the progeny of non-related individuals. The fitness of a population decreases as the homozygosity increases. The ability of a population to contribute to future generations is also called fitness (Stearns, 1992). The opposite of inbreeding is heterosis or hybrid vigour. The fitness lost through inbreeding can be improved by crossing. Cross breeding reduces the loss of alleles and increases fitness through increased heterozygosity (Falconer and MacKay, 1996). The production of interspecies hybrid abalone can potentially provide production gains. Heterosis or hybrid vigour in crossbreeds may also improve desirable traits like growth (Arai and Okomura, 2012). All possible combinations of *Haliotis hannai, Haliotis gigantea* and *Haliotis madaka* was performed in Japan by Ahmed *et al. (2008).* It concluded that inter-specific hybridization is possible in abalone. Crosses between *H. hannai* and *H. madaka* were easier to achieve than any crosses involving *H. gigantea.* Through histological studies it was confirmed that the hybrids can produce viable gametes. This suggests that hybridisation can be used in selective breeding strategies to improve growth rates.

Ploidy manipulation is probably the most researched avenue of abalone genetics. The potential advantages are the production of infertile animals and faster growth. Triploidy is induced through

inhibition or expulsion of the first or second polar bodies after fertilization of the egg. The induction is achieved by temperature shock, pressure shock, use of cytochalasin B, caffeine or 6-demethylaminopurine. The success of the induction rates vary among abalone species. Triploidy induction in *H. rufescens* doubled the average growth rate whereas no significant increase in growth rates was observed in *H. discus hannai* (Elliot, 2000). A study by Schoonbee (2008) on the effect of triploidy on the growth of South African indigenous abalone, *H. midae,* provided no convincing evidence of faster growth of juvenile abalone up to two years of age.

Abalone breeding can benefit from modern molecular techniques such as gene manipulation and transgenesis since it has a long generation interval and it may take a long time before the effects of conventional selective breeding programmes are seen. Gene transfer technology can potentially provide large genetic gains much faster than traditional breeding approaches which may take several generations. Abalone have growth hormone-like molecules which makes it a candidate for the manipulation of growth hormones. Growth increases of 50- 100% have been reported in abalone using gene transfer. The technology has tremendous scope for improvement. There is however public concern about any product that was genetically enhanced, as well as issues about the intellectual property and safeguarding of the strains and products (Elliot, 2000).

2.5 Genetic variation in wild and cultured populations

The population genetic structures of abalone are subject to conservation of wild populations and the increased production of cultured populations. A study by Van der Merwe (2009) was aimed at assessing the population genetic structure of wild populations of indigenous South African abalone, *H. midae.* Samples of abalone were collected for a timespan of five years along the geographical range of the species in South Africa. Microsatellite and single nucleotide polymorphism (SNP) markers were used to assess the degree of gene flow in nine wild abalone populations from different regions. High numbers of microsatellite markers have been developed for several species (Rhode and Roodt-Wilding, 2011). Initially eight microsatellite markers were identified and used to investigate the levels of allelic variation within and between the nine locations. The study found moderate to high levels of genetic variation amongst the nine populations of *H. midae*. The high level of microsatellite variation is reflective of a large effective population size of species in a marine environment. The study found the inbreeding coefficient

(F_{IS}) within the populations to be low, while the differentiation between the populations (F_{ST}) is high.

The effective populations size (N_{Θ}) is used as a parameter to monitor population demographics and is especially important in populations that are rapidly declining and susceptible to bottleneck effects, as could be the case with *H. midae* due to over exploitation. The microsatellite results in the study suggests effective population expansion rather than decline.
In conjunction with the eight microsatellite markers, twelve SNP markers were used to corroborate the microsatellite data. The SNP data reflected the subtle genetic differentiation between the East and West coast populations with a hydrogeographic barrier near Cape Agulhas (Van der Merwe, 2009).

Rhode (2013) studied the evolutionary forces influencing the divergence in *H. midae* populations by using population genomics. F_1-cultured animals were collected from three facilities, on from the east, the west and south coast of South Africa. The abalone were selected at random to reflect the F_1-population of each respective facility. This study used 150 microsatellite markers developed for *H. midae,* compared to only eight by Van der Merwe (2009). No significant differences were observed in genetic diversity across all the populations, wild and cultured, based on the number of alleles.

Pairwise F_{ST}-tests for genotypic differences in populations estimated highly significant differences between wild and cultured populations and amongst cultured populations (Rhode, 2013).

2.5.1 Genetic variation in the rerformance recording scheme populations

This current study is based on populations entered into a performance recording scheme (PRS). A molecular genetics study was done on these populations to investigate the genetic status of the populations in relation to each other and the wild populations from which the broodstock populations were sourced from. Swart (2012) conducted parentage assignment by use of 12 species-specific microsatellite markers on these. The faster-growing PRS abalone were sampled. The microsatellite data made it possible to assess the influence of selection on genetic diversity of the populations as well as the genetic differentiation between and within wild and cultured populations.

The results confirmed that broodstock used to produce the PRS populations was an accurate representation of the wild populations as no significant differentiation was found between the PRS broodstock and the wild populations they were sourced from. The genetic diversity levels in the PRS populations were similar to wild populations. Through parentage assignment it was found that there was an uneven contribution by PRS broodstock. This was expected as variation in parental contribution is common in broadcast spawning molluscs (Slabbert *et al.*, 2009). The broodstock of two of the participating hatcheries (Abagold (Pty) Ltd and Aquafarm Development (Pty) Ltd) contributed only 32% and 37% to the spawning event respectively and were the only two farms displaying loss in genetic diversity in the F_1-population. Varying contribution levels were found over all the broodstock used in this study.

All the cultured offspring populations that were used in this study displayed significant differentiation from their respective broodstock populations. This can be attributed to artificial selection as only the faster-growing offspring were used during parentage assignment, as well as the differential contributions of broodstock (Swart, 2012).

2.6 Summary

South African indigenous abalone, *H. midae,* are univalve marine gastropods found along the coastline. It is the largest of South African species and the only one commercially exploited. They reproduce through broadcast spawning. After fertilisation a complex larval stage is followed by settlement and development to adult abalone. Several factors and physiological aspects impact on growth and growth rate of the juvenile abalone.

Very little evolvement has occurred in the genetic improvement of abalone. The development of domesticated and genetically improved strains is crucial to maintaining *H. midae* status on the international market. Genetic variation and correlation are the building blocks of a selective breeding programme. The use of molecular techniques and technology in conjunction with quantitative strategies can greatly enhance genetic breeding programmes.

Genetic differentiation exists between broodstock- and offspring populations and among the offspring populations used in this study. This can be attributed to the effect of selection and the differential contribution by the broodstock.

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Chapter 3

Materials and methods

Chapter Three: Materials and Methods

3.1 Materials

3.1.1. Entering animals into study

A consortium was formed amongst five commercial abalone farms to participate in the selective breeding program. The five farms are located in the Walker Bay region of the Western Cape. Abagold (Pty.) Ltd, Aquafarm Development Company (Pty.) Ltd. and HIK Abalone (Pty.) Ltd are situated in Hermanus. Irvin and Johnson Ltd is located at Danger Point and Roman Bay Sea Farm (Pty.) Ltd is near Gansbaai.

Each farm participating at the time, had a set of active broodstock of approximately 150 animals that were randomly collected from the surrounding wild populations and conditioned for artificial spawning over a period of time. A spawning regime was established amongst the farms whereby each farm conducted a synchronised mass spawning on a set date, each generating a particular set of offspring.

Progeny groups were reared at these locations through the larval, settlement and weaning stages, to an age of 6 months. Each farm followed its own husbandry protocols in relation to handling, housing, feeding, grading, densities, etc. These protocols are fairly standardised and production parameters were largely similar across the five farms, though with some level of environmental variance.

3.1.2 Identification and tagging

Each participating hatchery subsequently provided a random sample of 3000 F_1 animals from the synchronized spawning to be entered into a performance recording scheme (PRS). These animals were randomly selected from mass spawned offspring and tagged as per location once they had reached an size of 6-10 mm at age six months. Animals were anesthetised with $MgSO_4$ to allow easier handling. Initial tagging was done with silicone tubes. The tubes were cut into triangles and inserted through a breathing hole of the abalone shell (Figure 3.1). This method proved relatively

unsuccessful. Inserting the triangular tag too deep led to irritation of soft tissue of the abalone and caused mortalities. Too little insertion of the tag led to it dislodging.

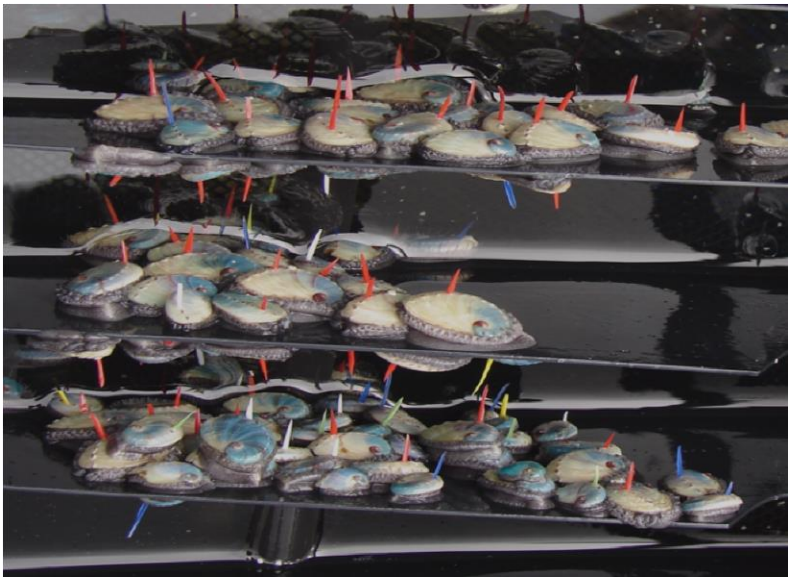

Figure 3.1 Silicone tube tagging method implemented to identify cohorts of abalone (*H. midae*) (Photograph, Prof. D. Brink).

Tag loss became visible after a period of 4 weeks after which all the identifiable animals were retagged using colour coded bee tags. The factors leading to tag loss were considered and found to be of a random nature and is discussed in the results section. The tags are manufactured for the honey bee industry by Swienty® in Denmark. It is small (3 mm in diameter) and marked with a number from 1-99. The numbering of the tags carries no significance in this study. After gently drying the shell of the abalone, the bee tag was simply glued onto the shell using Super Glue® (Figure 3.2). Each participating farm was represented by a different colour bee tag.

Figure 3.2 Bee tag tagging method used to retag identifiable animals after tag loss and mortalities.

3.2 Experimental design

The experimental design presents that of a balanced block design. The tagged, subscribed animals (n=3000) were randomly divided into five groups which represents the participating hatchery breeding cohort (referred to as "cohort" hereafter). The five groups were then randomly divided into three replicates and put into mesh bags for transport and assigned to a participating farm or rearing location (referred to as location)

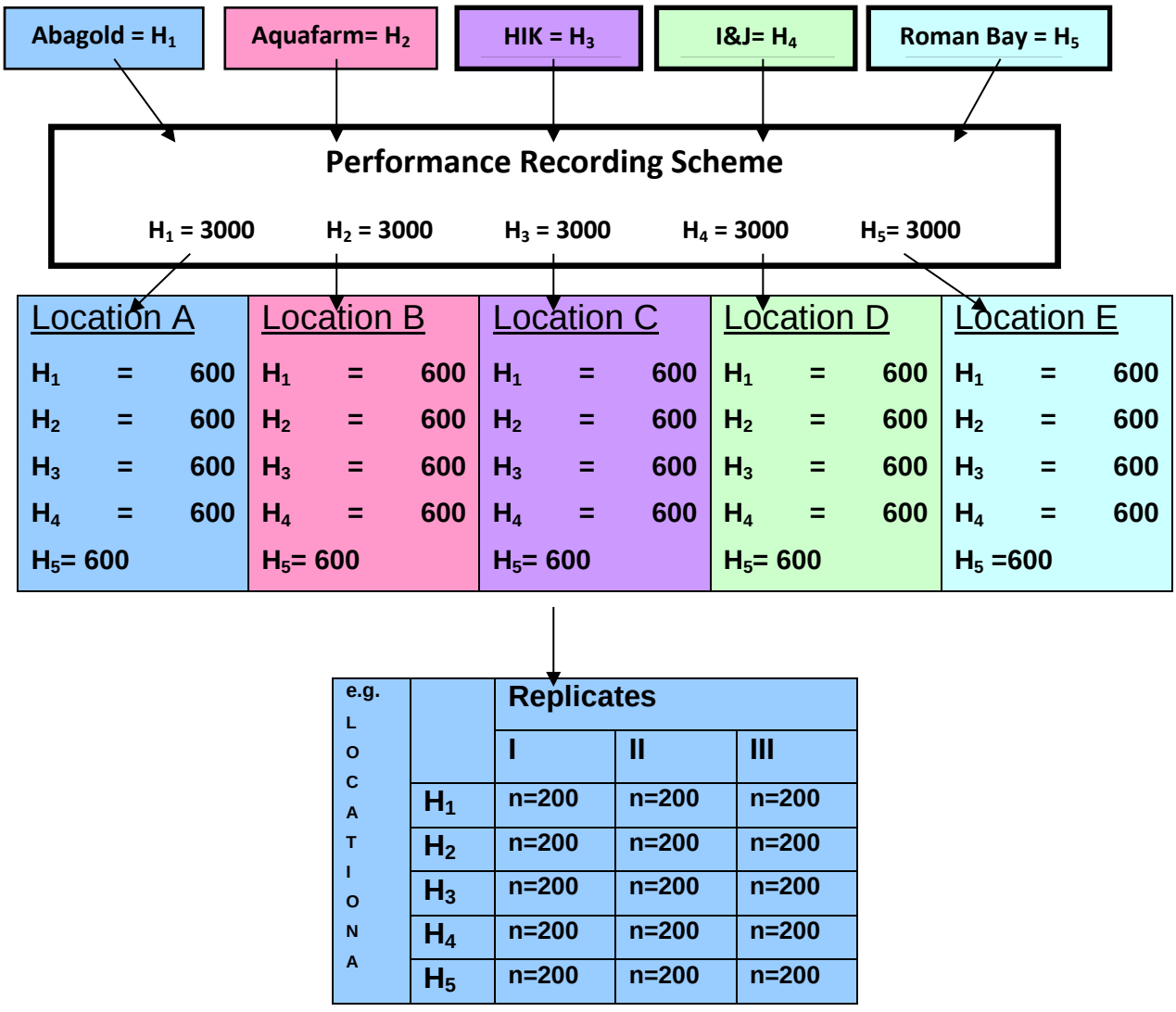

Figure 3.3 Performance Recording Scheme design.

All five cohorts are represented at each location containing three replicates with each replicate consisting of a random selection of 200 animals from each of the five hatcheries (Figure 3.3). Each replicate, containing all five cohorts, was reared in the same basket, standardising environmental conditions amongst the cohorts and thereby environmental variance (Figure 3.1).

3.3 Growth measurements

The subscribed PRS animals at each location underwent a 30 day adaptation period, before growth measurements commenced. At the age of 9 months all animals in a particular replicate were anaesthetized using either $MgSO_4$ or CO_2 depending on the practices of the particular farm. The anaesthetized animals were transferred to mesh baskets to allow excess water to drain. Random samples of each replicate were measured at three-monthly intervals over a period of 21 months. A sample size of 16 was considered as adequate as it is a number at which the standard error of means is suitably reduced while still being practically feasible (see Addendum A). Randomised samples were obtained by spreading the anaesthetized animals on a table and spanning a rope diagonally across them. Starting from one side, the first 16 animals of each of the five cohorts touching the rope were selected as the randomised sample (Fig 3.4). The shell length was measured to the nearest mm with a Waldo 712-140 digital caliper as the distance from the anterior to the posterior extremities. Body weight was recorded in grams with an UWE JW-500 electronic scale.

Figure 3.4 Randomised selection method (Photograph, Prof. D. Brink).

3.4 Definition of traits and statistical analysis

The estimations of growth rates for both length and weight gain required the measurement of shell length and wet body weight at each age interval. Since the weight to age curve followed a non-linear, quadratic curve the weight plots were log-transformed (log_{10}) using R version 2.15.2 (R Core Team, 2012) to fit a linear model. The growth rate of weight was calculated as the regression coefficient (b_w) of the regression of log_{10} sample mean weight on age. The growth rate of length that followed a linear curve was calculated as the regression coefficient (b_l) of the regression of sample mean length on age.

The data from the eight sets of measurements were entered into Microsoft® Office Excel® manually and converted into a format compatible with Statistical Analysis System Enterprise Guide 9.1 (SAS Institute Inc. 2012) and T version 2.15.2 (R Core Team, 2012). Statistical analysis was done to assess the differences between growth rate regression coefficients of the five cohorts and five locations. Additionally, assumption testing of all models was conducted by means of normality testing of model residuals utilising the Shapiro-Wilk test, homogeneity of variances was tested by means of Bartlett's test for homoscedasticity and interaction between main effects was tested utilising interaction models described below. The significance threshold for all hypothesis testing was set at P=0.05. Where significant differences were detected for main effects, multiple pairwise comparisons were carried out with Bonferroni p-adjustment method to evaluate and compare the cohorts and locations.

Additional data cleaning steps included testing for outliers by means of 1.5 the inter-quartile range, sub setting data to omit latter measurements affected by tag losses and the omission of incomplete records for cohorts or locations.

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3.5 Description of models

A two-way analysis of variance (ANOVA) was performed on the full model using the General Linear Models (GLM) procedure of SAS 9.1, to assess the effect of genotype and location on the growth rate coefficients. An interaction term was added to evaluate whether there was interaction between the main effects.

The following model was fitted for the main effects and interaction term:

$$y_{ijk} = \mu + \pi_i + \theta_j + \theta\pi_{ij} + \varepsilon_{ijk}$$

where, y_{ijk} is the regression coefficient of mean length or $\log_{10}$ mean weight on age of the k^{th} replicate, of the i^{th} cohort at the j^{th} location; μ is the overall mean; π_i is the effect of the i^{th} cohort, θ_j is the effect of the j^{th} location and $\theta_{\pi ij}$ is the effect of the interaction between the i^{th} cohort and j^{th} location; ε_{ijk} is the random residual error of the mean length or mean weight of the k^{th} replicate, of the i^{th} cohort at the j^{th} location.

Where no interaction was detected, the analysis of variance analysis was carried out on the following model:

$$y_{ijk} = \mu + \pi_i + \theta_j + \varepsilon_{ijk}$$

where, $y_{ijk}$is the regression coefficient of mean length or $\log_{10}$ mean weight on age of the k^{th} replicate, of the i^{th} cohort at the j^{th} location; μ is the overall mean; π_i is the effect of the i^{th} cohort, θ_j is the effect of the j^{th} location; ε_{ijk} is the random residual error of the mean length or mean weight of the k^{th} replicate, of the i^{th} cohort at the j^{th} location

Where initial length or weight was added as a covariate in the two-way analysis of variance, the following equation was fitted to the model:

$$yijk = \mu + b(\alpha ijk) + b(\beta ijk) + \pi i + \theta j + \theta\pi ij + \varepsilon ijk$$

where, y_{ijk} is the mean length or mean weight of the k^{th} replicate, of the i^{th} cohort at the j^{th} location; μ is the overall mean; $b(\alpha_{ijk})$ is the regression of age of the k^{th} replicate, of the ith cohort at the j^{th} location on mean length or mean weight ; $b(\beta_{ijk})$ is the regression of the initial mean length or initial mean weight of the k^{th} replicate, of the i^{th} cohort at the j^{th} location on mean length or mean weight ; π_i is the effect of the i^{th} cohort, θ_j is the effect of the j^{th} location and $\theta_{\pi ij}$ is the effect of the interaction between the i^{th} cohort and jth location ; ε_{ijk} is $_{the}$ random residual error of the mean length or mean weight of the k^{th} replicate, of the i^{th} cohort at the j^{th} location.

3.6 Summary

Five commercial abalone farms each entered 3000 randomly chosen animals obtained from synchronised mass spawning of conditioned broodstock into a performance recording scheme. The five cohorts were assessed over the five locations represented by three replicates per location with 200 randomly assigned animals per replicate. Shell length and body weight were measured every three months over a period of 21 months. The collected data were entered into Microsoft® Excel® to be converted into a compatible format for further analysis in SAS® Enterprise Guide® and R version 2.15.2 (R Core Team, 2012). Statistical analyses were applied to the data to assess interaction between the main effects (i.e. cohort and location). When no interaction was detected, the statistically significant differences between cohorts and between locations was analysed. Pairwise test were carried out to further analyse the differences. Equations were fitted to the models to describe the effects contributing to the observed variance.

3.7 References

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Addendum A

Extract from Notes for Biometry 212, University of Stellenbosch

4. Formula for the standard error of the mean

Calculation of the standard deviation of an estimator, like $\bar{x}$ above, with the aid of repeated sampling is tedious and usually impossible. This quantity can however also be estimated from a single observed sample and is known as the *standard error* of the estimator.

Suppose that we have taken a sample consisting of the observations $x_1, x_2, x_3, \ldots, x_n$ from a population. Subsequently we estimate the population mean, μ, with the sample mean, $\bar{x}$, and then calculate the sample variance, s^2. In order to estimate the standard error of the sample mean, $\sigma_{\bar{X}}$, from the formula given in the following theorem, we assume that the observations are all *equally precise* (have the same precision, or that the measurement occurred with the same variation) and they are *independent* of each other so that their covariances = 0.

Definition

We say the r.v.'s $X_1, X_2, X_3, \ldots$ are *homoscedastic* if $\text{var}(X_1) = \text{var}(X_2) = \text{var}(X_3) = \cdots$.

Otherwise we term them *heteroscedastic*. Homoscedasticity gives statistical meaning to the idea of "equally precise".

Theorem The variance and standard error of the mean of uncorrelated and equally precise observations from a population

If $X_1, X_2, X_3, \ldots$ are uncorrelated r.v.'s with common variance σ^2,

then $\sigma^2_{\bar{X}} = \dfrac{\sigma^2}{n}$, that is to say, the standard error $\sigma_{\bar{X}} = \dfrac{\sigma}{\sqrt{n}}$.

We will prove this theorem in a following note set (NS 15: Bienaymé's theorem).

The term "*standard error*" is synonymous with "*standard deviation*" in the sense that both refer to the square root of a variance. The difference lies in the circumstances in which the term is applicable. The standard deviation refers to the degree of dispersion of individual observations in the population or sample, while standard error refers to the degree of dispersion associated with the estimator, i.e. the variety of values that the estimator will assume with *consecutive estimates*. We thus define the standard error as the standard deviation of the *population of estimates* originating from that estimator.

A further result now comes to the fore, namely that the standard error of the estimate becomes smaller with an increase in the number of observations included in the sample. We can interpret this as an increase in the precision of estimation, so that the standard error can be interpreted as a

measure of the accuracy of the relevant estimator. In this sense we define *accuracy* as the quantity by which our estimate differs from the true population value.

The following results of an experiment demonstrate this result.

Suppose an infinite population, consisting of the numbers 0, 1, 2, …, 9 in equal proportions. Define r.v. X = random number between 0 and 9. Thus:

The probability distribution of X = random number between 0 and 9:

x	0	1	2	3	4	5	6	7	8	9	Total
$P(X = x)$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	1

Now the population mean $\mu = E(X) = \frac{1}{10}(1+2+...+9) = 4.5;$

Also $E(X^2) = \frac{1}{10}(0^2) + \frac{1}{10}(1^2) + ... + \frac{1}{10}(9^2) = 28.5$

and the population variance $\sigma^2 = E\left(X^2\right) - \left[E(X)\right]^2 = 28.5 - (4.5)^2 = 8.25$

so that the standard deviation $\sigma = \sqrt{8.25} = 2.875.$

Each of 48 students from the 1978 Biometry 214 class carried out the following instructions, according to which they repeatedly estimated the value of μ with a different number of observations from the population of random numbers between 0 and 9:

$n = 1$:	Read a number (x_1) from a table of random numbers and write down the result.
$n = 4$:	Read 3 more numbers (x_2, x_3, x_4) and write down the average of x_1, x_2, x_3, x_4.
$n = 9$:	Read 5 more numbers and write down the average of x_1, x_2, …, x_9.
	Also obtain the mean of samples of sizes $n = 16$, $n = 25$, and $n = 36$.

Each student naturally had a different starting point in the table so that there are 48 individual means for each n value.

The standard errors for the different sized samples above are as follows:

n = 1	$\sigma_{\bar{x}} = \frac{2.875}{\sqrt{1}} = 2.875$
n = 4	$\sigma_{\bar{x}} = \frac{2.875}{\sqrt{4}} = 1.436$
n = 9	$\sigma_{\bar{x}} = \frac{2.875}{\sqrt{9}}$ = 0.957
n = 16	$\sigma_{\bar{x}}$ = 0.718
n = 25	$\sigma_{\bar{x}}$ = 0.574
n = 36	$\sigma_{\bar{x}}$ = 0.479
n = 49	$\sigma_{\bar{x}}$ = 0.411
n = 64	$\sigma_{\bar{x}}$ = 0.359

The most obvious result is that the standard error becomes smaller with an increase in the sample size, *n*. Note that an increase in the number of observations initially has a large effect on the size of the standard error, but that the effect is minimal when the number of observations exceeds 16. When *n* increases from 1 to 4 the standard error is halved. The same thing happens *n* is increased from 4 to 16, but the following halving only occurs at *n* = 64.

The decrease in standard error with the increase in sample size is reflected by the spread of the 48 means around μ that diminishes as *n* increases (Diagram 2). The dotted lines in the diagram indicates a distance of one standard error on both sides of μ:

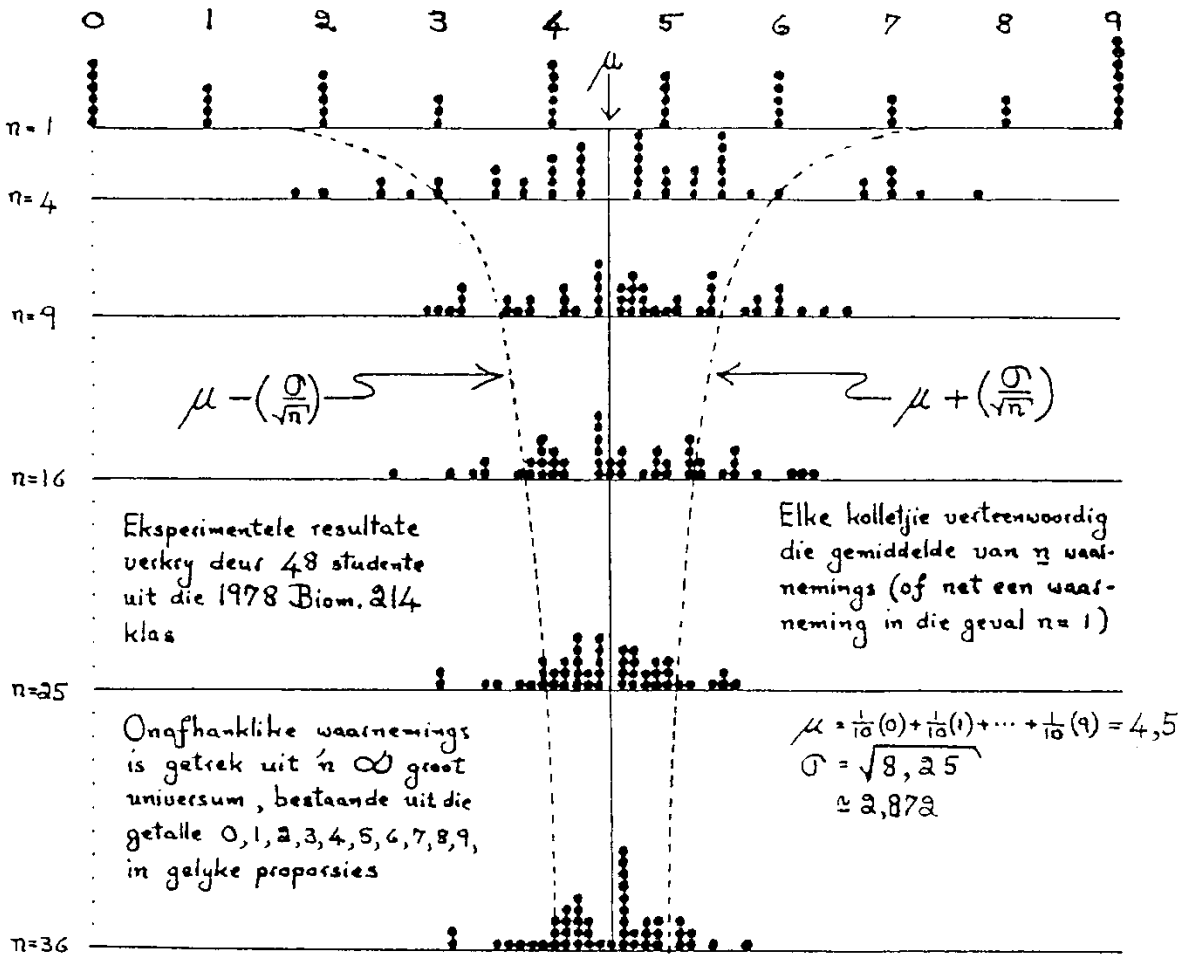

DIAGRAM 2: An experimental demonstration that $\mu_{\bar{x}} = \mu$ and that $\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$

Chapter 4

Results and analysis

Chapter Four: Results and Analysis

Growth data was collected in the form of individual body weight (weight) and individual shell length (length) every three months over a period of 24 months starting at an age of approximately ten months. During the latter stages of the study some limitations regarding tag loss and farm management error came to light. These were considered during analysis and will be discussed in a progressive manner as follows:

4.1 Results and analysis of full model

4.2 Results and analysis of data corrected for progressive tag loss

4.3 Results and analysis of data corrected for progressive tag loss and farm management error

4.4 Results and analysis of data corrected for differences in initial size of cohorts

4.1 Results and Analysis of full model

The main objectives of the study were to assess whether there is a difference between the five breeding populations or breeding cohorts and the geographical locations in terms of growth measure as both weight and length gain. There was also the distinct possibility of interaction between cohort and location or environment.

Initial plotting of the full data set revealed that the length growth plot followed a clear linear trend (Figure 4.1) whereas weight growth was clearly non-linear (Figure 4.2). A quadratic equation was therefore fitted to the weight growth plots.

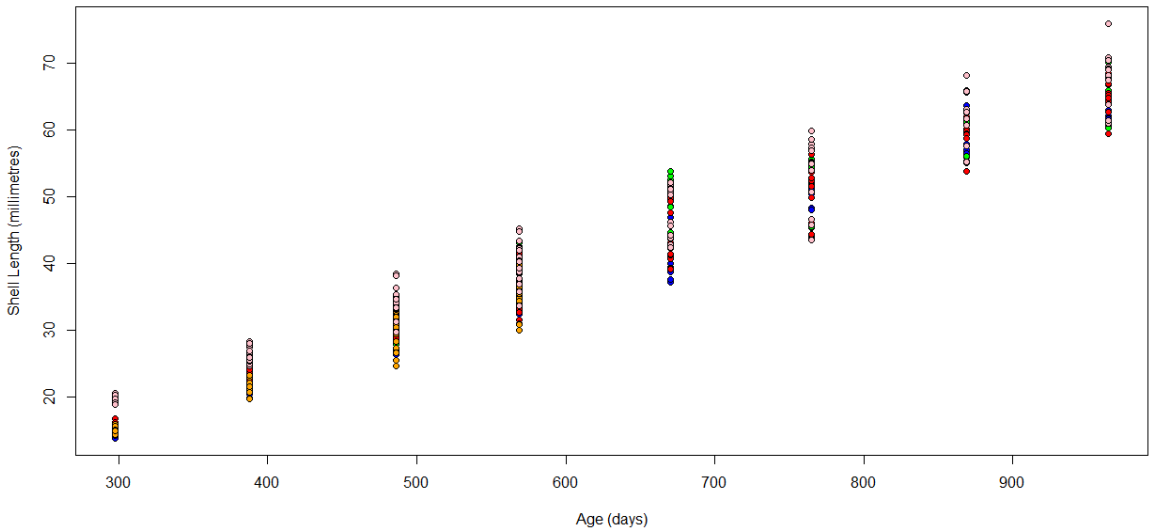

Figure 4.1 Average length over age of all cohorts at all locations.

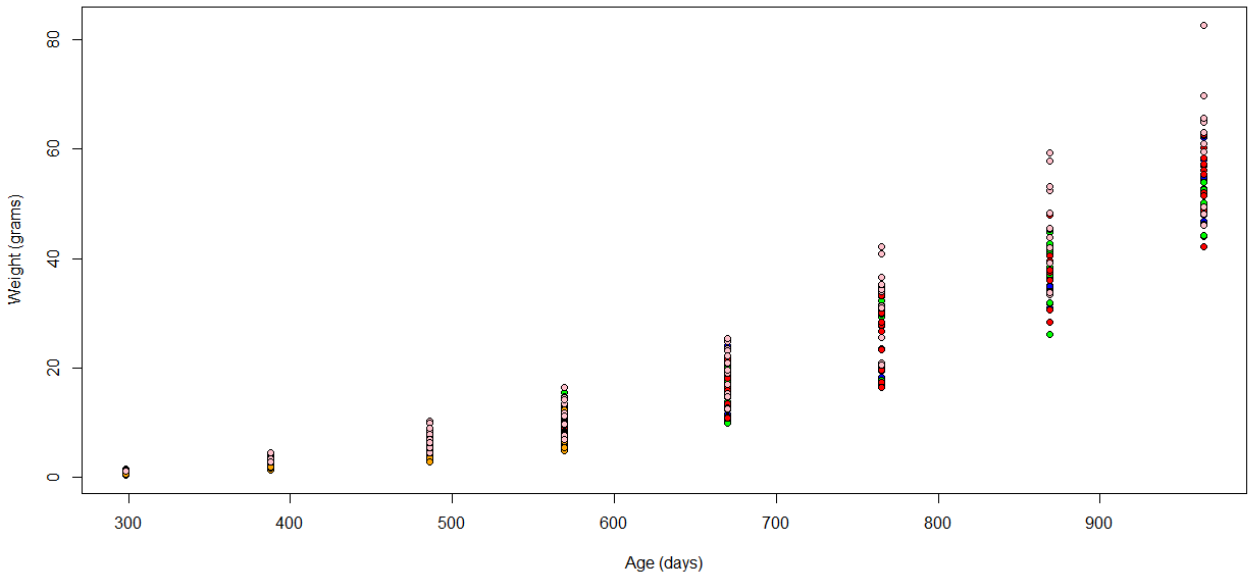

Figure 4.2 Average weight of all cohorts at all locations.

The data was tested for normality and homoscedasticity. After the log transformation of the weight growth data, the data was tested for normality of the model residuals using the Shapiro-Wilk test and was found to be well within the bounds of the normality assumption of analysis of variance (ANOVA) (Shapiro and Wilk, 1965). The homoscedasticity assumption of analysis of variance was also met through Bartlett's test.

4.1.1 Analysis of variance of full model

Analysis of variance methods were used to determine if there were differences between the five breeding cohorts, the geographical locations and whether there was any cohort and location or environment interaction.

The null hypothesis tested in the ANOVAs was:

H_0: $\mu_1 = \mu_2 = \cdot\cdot = \mu_n$, where μ_i is the mean length or mean $\log_{10}$ of weight of the cohort or location.

The alternative hypothesis:

H_α: $\mu_1 \neq \mu_2 \neq \cdot\cdot \neq \mu_n$,

Since the weight growth plot follows a quadratic curve, the data were transformed to fit a linear model.

Table 4.1 ANOVA of mean weight of all measurements over all cohorts and locations over the series of eight sample measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-R*^{2}
(a) *Full model*					
Age	1	137383	12444.57	2.2e-16	0.966
Age^2	1	13047	1181.87	2.2e-16	
Initial Weight	1	1421	128.70	2.2e-16	
Cohort	4	119	10.80	2.18e-08	
Location	4	488	44.22	0.183	
Cohort x Location	16	398	25	0.0036	
Error	472	11			

This model captures a significant amount (96.6%) of the variation based on the adjusted R-square. It also detects interaction between cohort and location (P=0.036) making the interpretation of the differences between cohorts and locations impossible. The interaction refers to a change in rank order in terms of average growth rate and/or a change in the scale of differences. The rank order of the cohort populations from Aquafarm Development (Pty) Ltd, Irvin & Johnson Ltd and Roman Bay Sea Farm (Pty) Ltd changed over measurement intervals over the locations Irvin & Johnson Ltd and Roman Bay Sea Farm (Pty) Ltd in terms of both length gain (bl) and weight gain regression ($\log_{10}b_w$).

Table 4.2 ANOVA of mean length of all measurements over all cohorts and locations over the series of eight sample measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-R*^{2}
(a) *Full model*					
Age	1	126668	25077.20	2.2e-16	0.981
Initial Weight	1	1483	293.62	2.2e-16	
Cohort	4	85	16.86	6.19e-13	
Location	4	340	67.28	2.2e-16	
Cohort x Location	16	9	1.76	0.035	
Error	473	5			

As with the ANOVA of the mean weight the ANOVA of mean length the model captures a significant amount of the variation (adj. R^2=0.981) and also indicates interaction between cohorts and locations (P=0.035).

The project aims were expected to be satisfied through the analysis of the full model. Interaction between cohort and location does not allow for differences between cohorts and between locations to be interpreted. A progressive statistical process was implemented to investigate any possible factors contributing to the observed interaction between cohort and location.

4.2 Results and Analysis of model corrected for progressive tag loss

It was apparent during on farm sampling that tag loss was occurring. This could be due a number of reasons including encrusting organisms on the abalone shell masking the tags, human counting error, dislodging of tag during handling, mortality and failed adhesion of the tag. A progressive tag loss was observed at each sampling interval.

A study by Difford (2013) on the effectiveness of the use of an "internal reference" method within a combined family selection program used the same tagging method. The tag loss of the method used in this study was quantified and is represented in Table 4.3.

Table 4.3 Descriptive statistics of sample sizes due to tag loss through time (Difford, 2013).

Time since tagging (Months)	Mean number of tagged animals	Standard Deviation	Median	Count	Min.	Max.
0	15.93	0.277	16	173	14	16
6	15.59	1.42	16	173	2	16
12	15.05	2.88	16	173	2	16
18	12.65	4.26	15	173	0	16
24	8.83	4.65	9	173	0	16

It is important not to allow any form of bias. During cohort assignment 200 abalone per cohort were tagged and entered into each repeat at each location (N=200). A random sample of 16 abalone per cohort per repeat were measured (n=16). In some cases the progressive tag loss led to sample sizes of less than 10 (n<10) in multiple samples. This led to highly unbalanced sample sizes in the last two sets of measurements. The factors leading to tag loss therefore had to be

investigated for the introduction of bias. Through the observation of animals demonstrating tag loss no discernible bias could be detected. The factors considered to be leading to tag loss also display no discernible bias. It was therefore assumed that the factors leading to tag loss was random and analyses can be conducted accordingly. The tag loss was still significant and an area of weakness. Although it displays no form of bias the model of this study requires the use of mean measurements of samples and therefore it cannot be assumed that the samples are representative of the population. The significant decrease in sample size in the latter samples, with reference to the last two sets of measurements, decreases the accuracy of the regression analyses. It was decided to investigate the effect of it on the results of the full model by removing the last two data samplings.

4.2.1 Analysis of variance for model corrected for progressive tag loss

The weight growth data was log transformed to fit a linear model. Linear line regression (b-values) was used as an indicator of growth rate. It was regressed on age for each replicate as dependant variable over a series of six measurements or 18 months.

Table 4.4 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for log-transformed weight gain (b_w) over a series of six measurements (last two sets of measurements omitted).

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-*R^2
(a) *Interaction model*					
Cohort	4	3.71×10^{-6}	69.06	<0.001	0.83
Location	4	8.50×10^{-7}	15.80	<0.001	
Cohort x Location	16	1.30×10^{-6}	2.370	0.0105	
Error	50	4.59×10^{-7}			

The analysis for log-transformed weight regressed on age still displayed a significant interaction in relation to the random sampling of parent stock from the same geographical area between cohort and location (P=0.0105). The cohort populations from Aquafarm Development (Pty) Ltd displayed a significantly slower growth rate in terms of log-transformed weight gain regression at Abagold Ltd ($\log_{10}b_w = 0.0041 \pm 9.2 \times 10^{-5}$ log(grams) per day), and a significantly faster growth rate at Aquafarm Development (Pty) Ltd ($\log_{10}b_w = 0.0048 \pm 2.21 \times 10^{-4}$ log(grams) per day). A similar

observation was made for the cohort populations from Irvin & Johnson Ltd which displayed significantly slower growth rates in terms of log-transformed weight gain at Irvin & Johnson Ltd ($log_{10}b_w$=0.0032±1.65x10^{-5} log(grams) per day), and significantly faster growth rates at Roman Bay Sea Farms Ltd ($log_{10}b_w$=0.0038±3.27x10^{-5} log(grams) per day). Cohort populations from Roman Bay Sea Farms (Pty) Ltd performed significantly worse at Irvin and Johnson Ltd ($log_{10}b_w$=0.0027±2.04x10^{-5} log(grams) per day) than at the other four locations. Further analysis of the main effects model is therefore not possible (Table 4.4).

The analysis for length gain regressed on age showed no interaction between the main effects, cohort and location (P=0.4501) (Table 4.5). This allows for the main effects model to be considered and analysed.

Table 4.5 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements (last two sets of measurements omitted).

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-R*^{2}
(a) *Interaction model*					
Cohort	4	1.00x10^{-4}	5.54	0.0009	0.83
Location	4	1.31x10^{-3}	72.38	<0.001	
Cohort x Location	16	1.86x10^{-5}	1.02	0.4501	
Error	50	1.82x10^{-5}			

Table 4.6 Analysis of variance table for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-R*^{2}
(b) Main Effects Model					
Cohort	4	1.00x10^{-4}	5.54	0.0009	0.80
Location	4	1.31x10^{-3}	72.38	<0.001	
Error	66	1.83x10^{-5}			

The main effects model for cohort and location on growth regression coefficients for length gain indicated significant differences between the growth rates (b_l) for both main effects. The ANOVA indicates that a significant statistical difference was observed between the five cohorts over locations (P=0.0009) and locations over cohorts (P<0.001) in terms of length gain (b_l).

4.2.2 Pairwise testing of statistically significant differences

To further quantify and display the significant differences multiple pairwise t-tests were performed for both cohorts and locations in terms of length gain (b_w). The t-tests were Bonferroni adjusted. The pairwise differences between cohorts and between locations are displayed in Tables 4.7 and 4.10 respectively. The five cohorts and locations are hereafter represented by the following abbreviations:

Abagold Ltd:	ABA
Aquafarm Development (Pty) Ltd:	Aqua
HIK Abalone Farm (Pty) Ltd:	HIK
Irvin & Johnson Ltd:	I&J
Roman Bay Sea Farm (Pty) Ltd:	RB

Table 4.7 Pairwise differences between cohorts in terms of length gain (b_l) (Bonferroni adjusted).

Least Squares Means for effect Cohort

i/j	ABA	Aqua	HIK	I&J	RB
ABA		1.0000	1.0000	0.0146	1.0000
Aqua	1.0000		1.0000	0.0905	0.7868
HIK	1.0000	1.0000		0.3829	0.2115
I&J	0.0146	0.0905	0.3829		0.0003
RB	1.0000	0.7868	0.2115	0.0003	

P<0.05 considered as significant

The b_l-value is expressed in units of the average millimetre growth per day, or the average daily length gain (ADLG). This unit is easier to interpret when expressed as millimetre per month (mm/m).

Table 4.8 A t-test of the average daily length gain (b_l) of the five cohorts.

Cohort	Mean b_l-value	t-Grouping
I&J	0.07915	a
HIK	0.07585	a,b
Aqua	0.07495	a,b
Aba	0.07396	b
RB	0.07216	b

A further useful calculation is the expression of growth as the amount of days it will take for the abalone to reach marketable size, which is approximately 100 mm. This is a very useful tool for the farmer.

Table 4.9 Useful growth parameters of cohorts based on average length gain (b_l).

Cohort	Growth per month (mm/m)	Days to 100 mm
I&J	2.3744	1263
HIK	2.2754	1318
Aqua	2.2485	1334
Aba	2.2188	1352
RB	2.1648	1385

The ANOVA also indicates a significant statistical difference between the five locations (P<0.001) (Table 4.6). The same analyses can therefore be done on the pairwise differences between the five locations in terms of average length gain (b_l).

Table 4.10 Pairwise differences between locations in terms of daily length gain (b_l) (Bonferroni adjusted).

Least Squares Means for effect Location

i/j	ABA	Aqua	HIK	I&J	RB
ABA		<.0001	<.0001	0.0262	<.0001
Aqua	<.0001		0.0102	<.0001	0.0055
HIK	<.0001	0.0102		<.0001	1.0000
I&J	0.0262	<.0001	<.0001		<.0001
RB	<.0001	0.0055	1.0000	<.0001	

P<0.05 considered as significant

Table 4.11 A t-test of the average daily length gain (b_l) of the five locations.

Location	Mean b_l-value	t-Grouping
RB	0.08372	a
HIK	0.08341	a
Aqua	0.07805	b
Aba	0.06789	c
I&J	0.06300	d

As with the cohorts it is useful to convert these values into units that are more interpretable to farmers. The converted data is presented in Table 4.12 below

Table 4.12 Useful growth parameters of locations based on average length gain (b_l).

Location	Growth per month (mm/m)	Days to 100 mm
RB	2.5115	1194
HIK	2.5024	1198
Aqua	2.3414	1281
Aba	2.0364	1473
I&J	1.8900	1587

The pairwise comparative tests clearly displayed statistically significant differences between both the cohorts and locations. It is interesting to note that this was not expected as the cohorts were sampled from the same geographical area and general farming practices have become fairly standardised within the local industry over time with some level of differentiation in stocking density and feeding regimes. The farms are also located in the same geographical area. Three of the farms (Aquafarm Development (Pty) Ltd, HIK Abalone Farm (Pty) Ltd and Abagold Ltd) maintained a steady ranking order with Roman Bay Sea Farm (Pty) Ltd and Irvin & Johnson Ltd deviating from it. Roman Bay Sea Farm (Pty) Ltd performed best as location but produced the worst cohorts in terms of average length gain whereas Irvin & Johnson Ltd displayed the exact opposite, producing the best cohort but performing worst as location. These results must be considered, but there were other factors contributing to variance that will be discussed in later sections of the study.

4.3 Results and analysis of full model corrected for progressive tag loss and farm management error

The animals entered into the study were initially tagged by inserting a triangular silicone tag through the breathing pore of the individual abalone. Each of the five participating farms was assigned a colour and the animal tagged accordingly. Aquafarm Development (Pty) Ltd was assigned yellow tags. The yellow silicone tubes used to cut the triangular tags from was of a much harder, less elastic consistency than those assigned to the other four farms. This led to some difficulty properly lodging the tag in the breathing pore. The less elastic nature of the tag also led to it being caught on ridges in the housing systems and dislodging. These factors led to significant tag loss. After four sets of samplings it was decided to exclude Aquafarm Development (Pty) Ltd as a cohort on the basis of excessive tag loss and reduced sample size.

The participating farms were instructed each to follow their own on-farm protocol when housing and handling the animals in terms of the density of animals in each basket. The experimental animals however had to be kept apart from the commercial animals on the farm. The baskets containing the animals entered into the study were marked properly but it was still maintained under commercial operating conditions. A management error occurred at Abagold Ltd after the fifth interval that led to the experimental animals being size sorted together with the commercial batches. The random nature of these experimental groups and normality of data were distorted and could therefore no longer be considered for this study. Abagold Ltd was therefore eliminated as a location after five sets of measurements.

The aim of this study was to compare growth rates of the cohorts and locations. The regression of the growth rates over time was used to assess this and therefore Aquafarm Development (Pty) Ltd was still entered as a cohort and Abagold Ltd as location even though they only contributed four and five sets of measurements respectively. It is possible that these errors introduced a form of bias to the results. To investigate this possibility a further correction was applied to the data. Section 3.2 investigated the influence of the progressive random tag loss. The following analyses excludes the last two data sets (section 3.2) as well as Aquafarm Development (Pty) Ltd as cohort and Abagold Ltd as location altogether.

4.3.1 Analysis of variance of model corrected for progressive tag loss and farm management error

The weight plot follows a quadratic curve and was therefore log-transformed to fit a linear model. Linear line regression (b-values) was used as an indicator of growth rate. It was regressed on age for each replicate as dependant variable.

Table 4.13 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for log-transformed weight gain (b_w) over a series of six measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-*R^2
(a) *Interaction model*					
Cohort	3	9.08×10^{-7}	70.22	<0.001	0.87
Location	3	5.03×10^{-7}	38.89	<0.001	
Cohort x Location	9	1.44×10^{-8}	1.12	0.3789	
Error	32	1.29×10^{-8}			

The analysis for log-transformed weight regressed on age for the first time displays no significant interaction between cohort and location (P=0.3789) (Table 4.13) which allows further analysis and interpretation of the main effects.

Table 4.14 Analysis of variance table for main effects cohort and location on growth regression coefficients for log-transformed weight gain (b_w) over a series of six measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-*R^2
(b) *Main Effects Model*					
Cohort	3	2.73×10^{-6}	68.45	<0.001	0.87
Location	3	1.51×10^{-6}	37.91	<0.001	
Error	47	1.32×10^{-8}			

The ANOVA for the main effects model for log-transformed weight regressed on age indicates statistically significant differences in the regression coefficient, b_w, for both main effects, cohort and location (P<0.001) (Table 4.14).

Table 4.15 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-*R^2
(a) *Interaction model*					
Cohort	3	1.04×10^{-4}	10.48	<0.001	0.88
Location	3	1.12×10^{-3}	112.61	<0.001	
Cohort x Location	9	1.58×10^{-5}	1.59	0.1616	
Error	32	9.94×10^{-6}			

The ANOVA for length regressed on age indicates no significant interaction between cohort and location (P=0.161)(Table 4.15) allowing further interpretation of the main effects.

Table 4.16 Analysis of variance table for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements.

Source	d.f.	Mean square	*F*-ratio	*P*	*adj-*R^2
(b) *Main Effects Model*					
Cohort	3	2.37×10^{-6}	68.45	<0.001	0.87
Location	3	1.51×10^{-6}	37.91	<0.001	
Error	47	1.32×10^{-8}			

There is a statistically significant difference in the regression coefficient, b_l, for both main effects (P<0.001) as illustrated in Table 4.16.

4.3.2 Analysis of differences in log-transformed weight gain regression (b_w) between cohorts.

4.3.2.1 Pairwise testing of statistically significant differences in log-transformed weight gain regression (b_w) between cohorts

The pairwise differences in log-transformed weight gain, b_w, between cohorts are displayed in Table 4.17 using multiple Bonferroni-adjusted t-tests.

Table 4.17 Pairwise differences between cohorts in terms of log-transformed daily weight gain (b_w) (Bonferroni adjusted).

Least Squares Means for effect Cohort

i/j	Aba	HIK	I&J	RB
Aba		0.0646	0.0064	<.0001
HIK	0.0646		1.0000	<.0001
I&J	0.0064	1.0000		<.0001
RB	<.0001	<.0001	<.0001	

P<0.05 considered as significant

Table 4.18 A t-test of the average log-transformed daily weight gain (b_w) of the four cohorts.

Cohort	Mean $\log_{10}(b_w)$ value	t-grouping	Graph
I&J	0.0036	a	Figure 3.5
HIK	0.0035	ab	Figure 3.4
Aba	0.0034	b	Figure 3.3
RB	0.0030	c	Figure 3.6

The graphs representing the performance of each cohort over all locations are presented in Figures 4.3 to 4.6. Each graph is a representation of a cohort's average daily weight gain (ADWG) regressed over age over the four locations. The t-test values were converted into more interpretable and useful units and are presented in Table 4.19 below.

Table 4.19 Useful growth parameters of locations based on average log-transformed weight gain (b_w).

Cohort	Growth per month ($\log_{10}$(grams)/m)	Days to 100g
I&J	0.1069	876
HIK	0.1057	880
Aba	0.1019	912
RB	0.0889	1056

4.3.2.2 Graphical representation of statistically significant differences in log-transformed weight gain regression (b_w) between cohorts

Figures 4.3 to 4.6 display the performance of the cohorts over the four locations as the regression of ADWG over age in days. The growth rate is expressed in units of gram per day

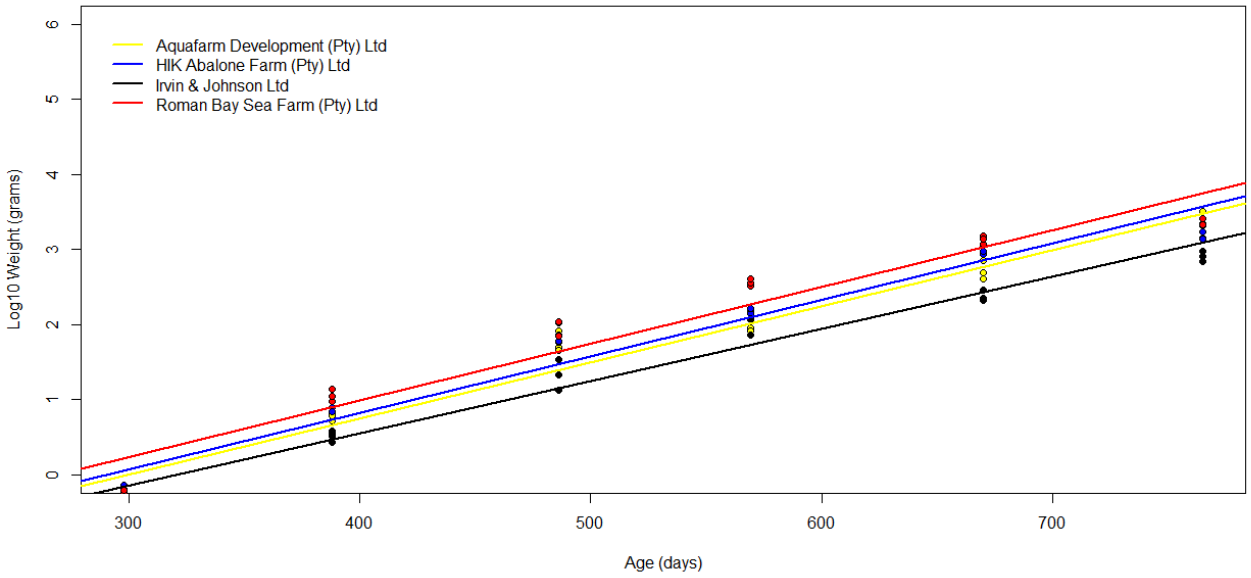

Figure 4.3 Log-transformed weight-wise growth regression (g/d) of cohort Abagold Ltd over four locations, over a period of 24 months.

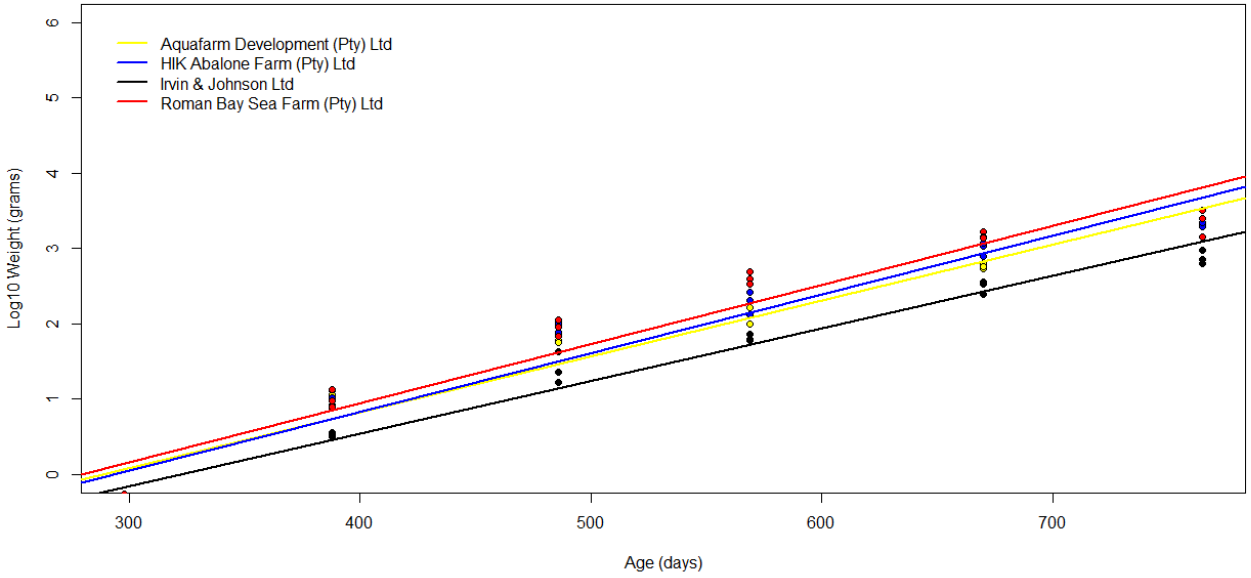

Figure 4.4 Log-transformed weight-wise growth regression (g/d) of cohort HIK Abalone Farm (Pty) Ltd over four locations, over a period of 24 months.

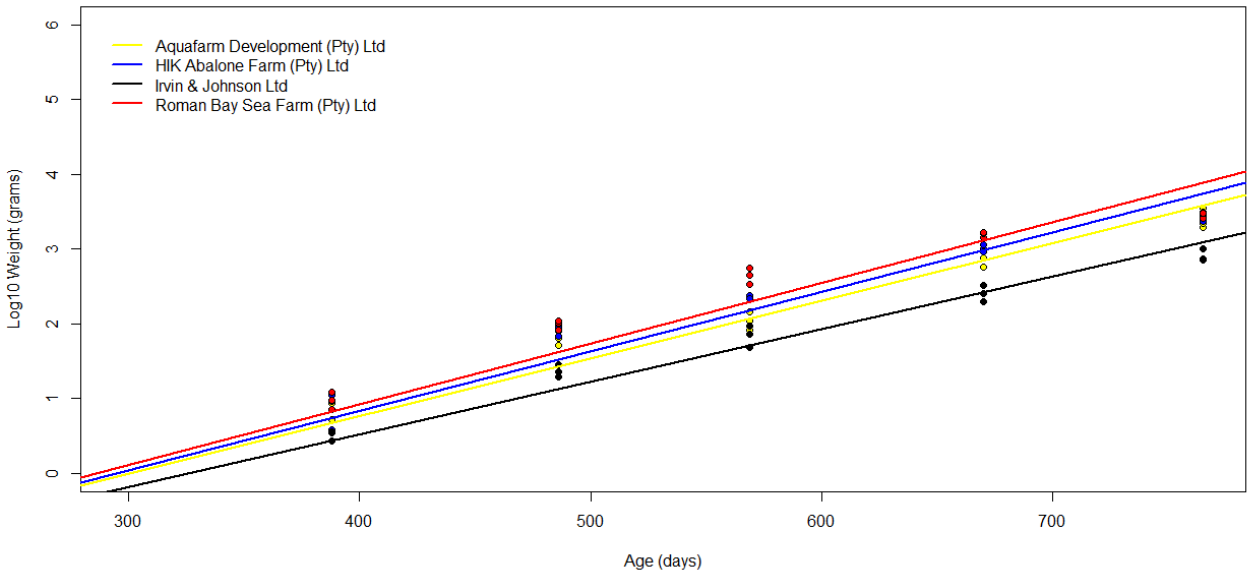

Figure 4.5 Log-transformed weight-wise growth regression (g/d) of cohort Irvin & Johnson Ltd over four locations, over a period of 24 months.

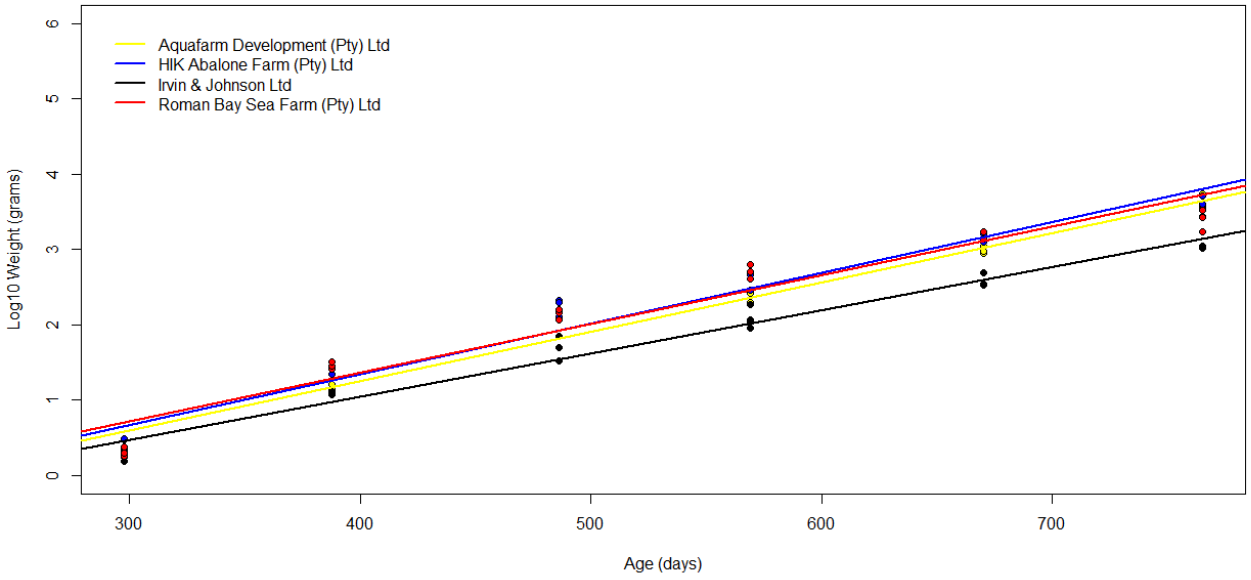

Figure 4.6 Log-transformed weight-wise growth regression (g/d) of cohort Roman Bay Sea Farm (Pty) Ltd over four locations, over a period of 24 months.

4.3.3 Analysis of differences in length gain regression (b_l) between cohorts.

4.3.3.1 Pairwise testing of statistically significant differences length gain regression (b_l) between cohorts

The pairwise differences in length gain, b_l, between cohorts are displayed in Table 4.20 using multiple Bonferroni-adjusted t-tests.

Table 4.20 Pairwise differences between cohorts in terms of daily length gain (b_l) (Bonferroni adjusted).

Least Squares Means for effect Cohort

i/j	Aba	HIK	I&J	RB
Aba		1.0000	0.0171	0.2899
HIK	1.0000		0.1383	0.0411
I&J	0.0171	0.1383		<.0001
RB	0.2899	0.0411	<.0001	

P<0.05 considered as significant

Table 4.21 A t-test of the average daily length gain (b_l) of the four cohorts.

Cohort	Mean b_l value	t-grouping	Graph
I&J	0.08066	a	Figure 3.9
HIK	0.07742	a, b	Figure 3.8
Aba	0.07631	b, c	Figure 3.7
RB	0.07353	c	Figure 3.10

The graphs representing the performance of each cohort over all locations are presented in Figures 4.7 to 4.10. Each graph is a representation of a cohort's ADWG regressed over age over the four locations. The t-test values were converted into more interpretable and useful units and are presented in Table 4.22 below.

Table 4.22 Useful growth parameters of cohorts based on average length gain (b_l).

Cohort	Growth per month (mm/m)	Days to 100mm
I&J	2.4198	1240
HIK	2.3226	1292
Aba	2.2893	1310
RB	2.2059	1360

4.3.3.2 Graphical representation of statistically significant differences in length gain regression (b_l) between cohorts

Figures 4.7 to 4.10 display the performance of the cohorts over the four locations as the regression of ADLG over age in days. The growth rate is expressed in units of mm per day

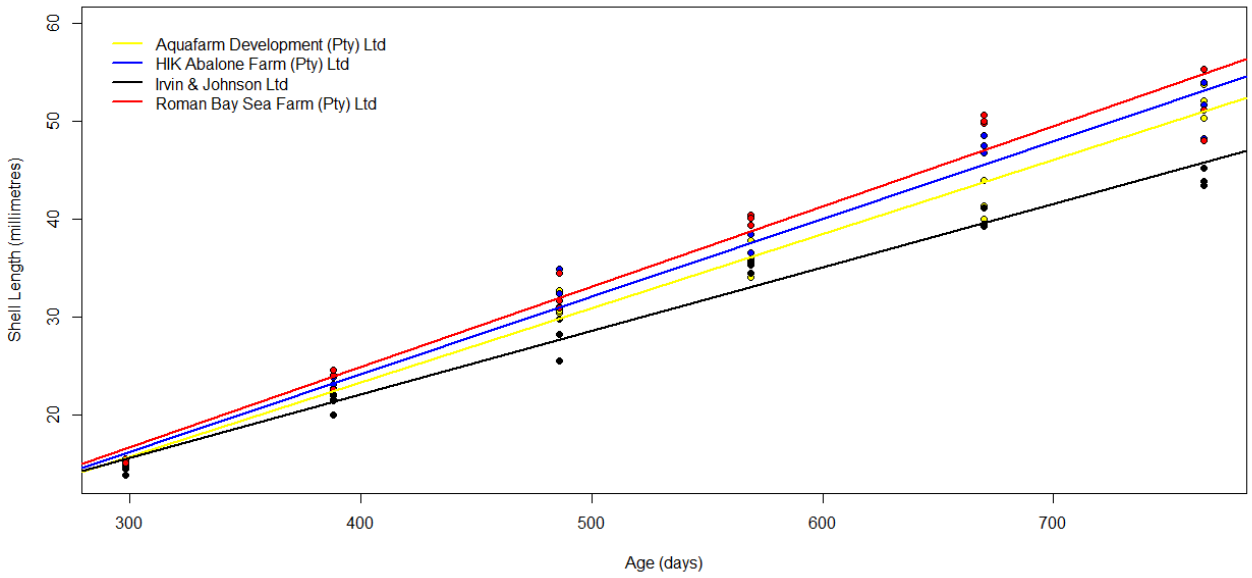

Figure 4.7 Length-wise growth regression (mm/d) of cohort Abagold Ltd over four locations, over a period of 24 months.

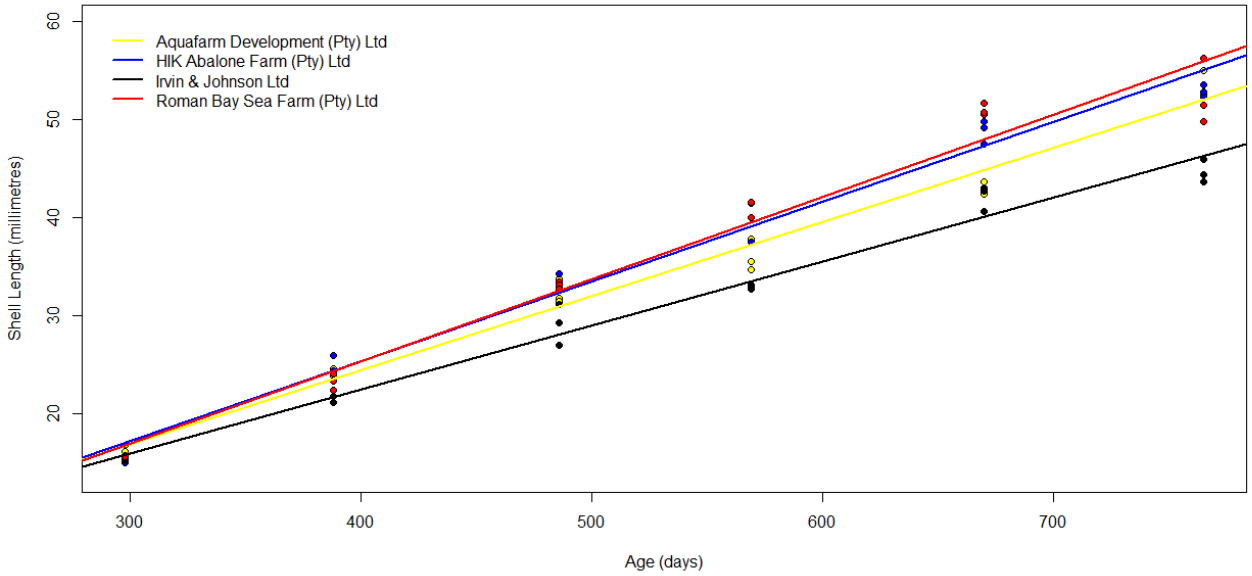

Figure 4.8 Length-wise growth regression (mm/d) of cohort HIK Abalone Farm (Pty) Ltd over four locations, over a period of 24 months.

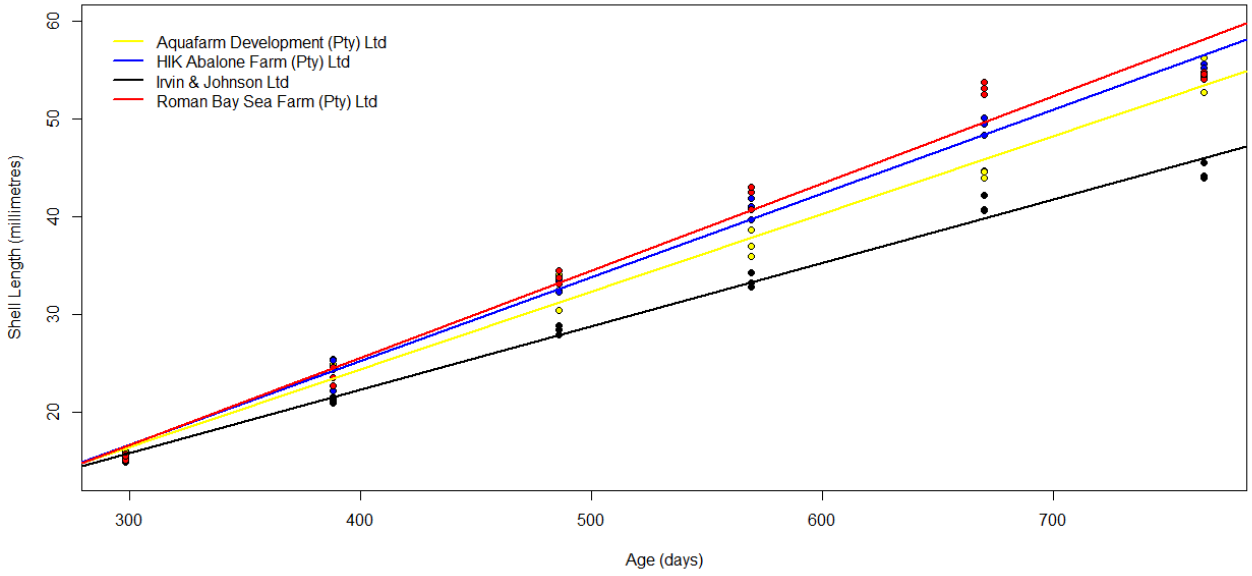

Figure 4.9 Length-wise growth regression (mm/d) of cohort Irvin & Johnson Ltd over four locations, over a period of 24 months.

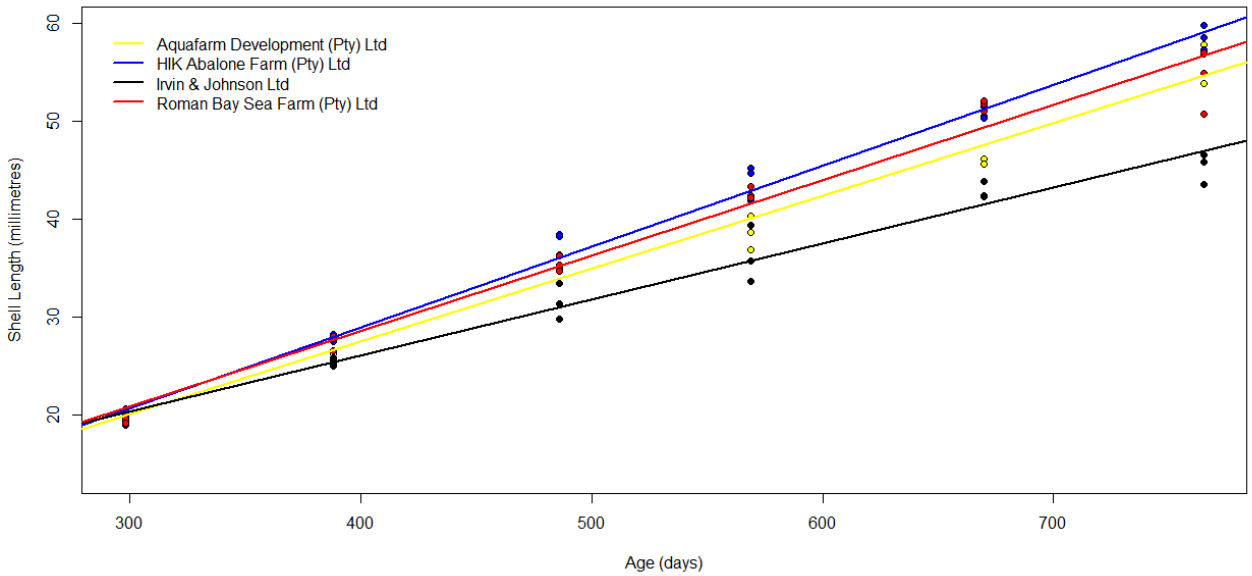

Figure 4.10 Length-wise growth regression (mm/d) of cohort Roman Bay Sea Farm (Pty) Ltd over four locations, over a period of 24 months.

4.3.4 Analysis of pairwise differences in log-transformed weight gain regression (b_w) between locations.

4.3.4.1 Pairwise testing of statistically significant differences in log-transformed weight gain regression (b_w) between locations

The pairwise differences in log-transformed weight gain, b_w, between locations are displayed in Table 4.23 using multiple Bonferroni-adjusted t-tests.

Table 4.23 Pairwise differences between locations in terms of log-transformed weight gain (b_w) (Bonferroni adjusted).

Least Squares Means for effect Location

i/j	Aqua	HIK	I&J	RB
Aqua		0.0469	<.0001	0.0118
HIK	0.0469		<.0001	1.0000
I&J	<.0001	<.0001		<.0001
RB	0.0118	1.0000	<.0001	

P<0.05 considered as significant

Table 4.24 A t-test of the average log-transformed daily weight gain (b_w) of the four locations.

Location	Mean log10 (b_w)value	t-grouping	Graph
RB	0.003517	a	Figure 3.14
HIK	0.003493	a	Figure3.12
Aqua	0.003363	b	Figure 3.11
I&J	0.003057	c	Figure 3.13

The graphs representing the performance of each cohort over all locations are presented in Figures 4.11 to 4.14. Each graph is a representation of a cohort's ADWG regressed over age over the four locations. The t-test values were converted into more interpretable and useful units and are presented in Table 4.25 below.

Table 4.25 Useful growth parameters of locations based on average log-transformed weight gain (b_w).

Location	Growth per month (log10(grams)/m)	Days to 100g
RB	0.1055	862
HIK	0.1048	876
Aqua	0.1009	900
I&J	0.0917	980

4.3.4.2 Graphical representation of statistically significant differences in log-transformed weight gain regression (b_w) between locations.

Figures 4.11 to 4.14 display the performance of the cohorts over the four locations as the regression of ADLG over age in days. The growth rate is expressed in units of grams per day.

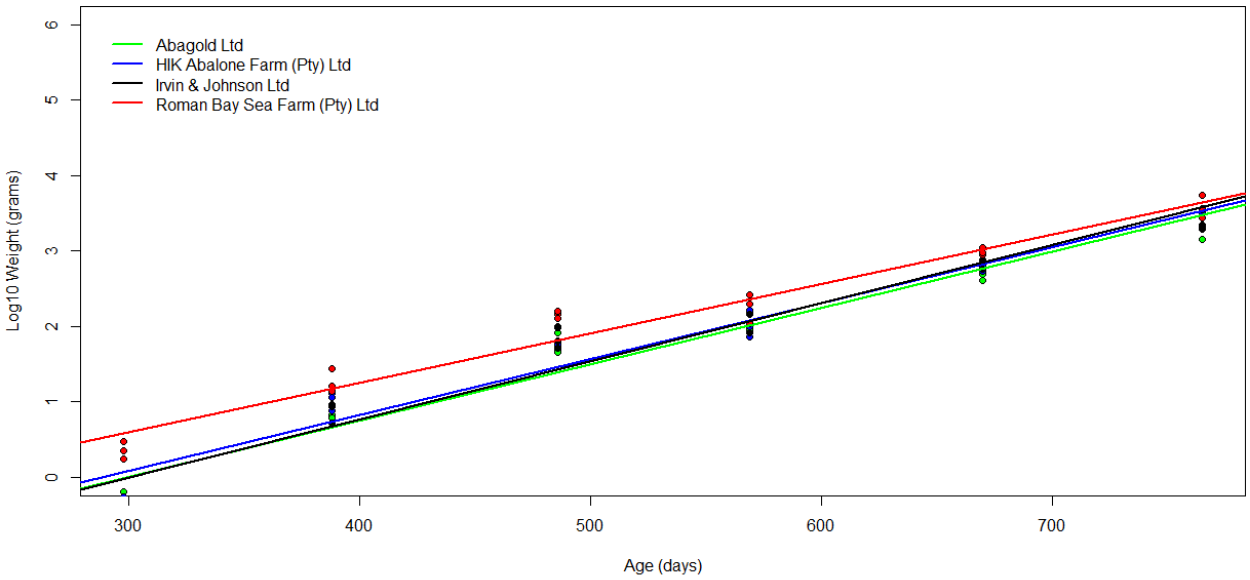

Figure 4.11 Weight-wise growth regression (g/d) of location Aquafarm Development (Pty) Ltd over four cohorts, over a period of 24 months.

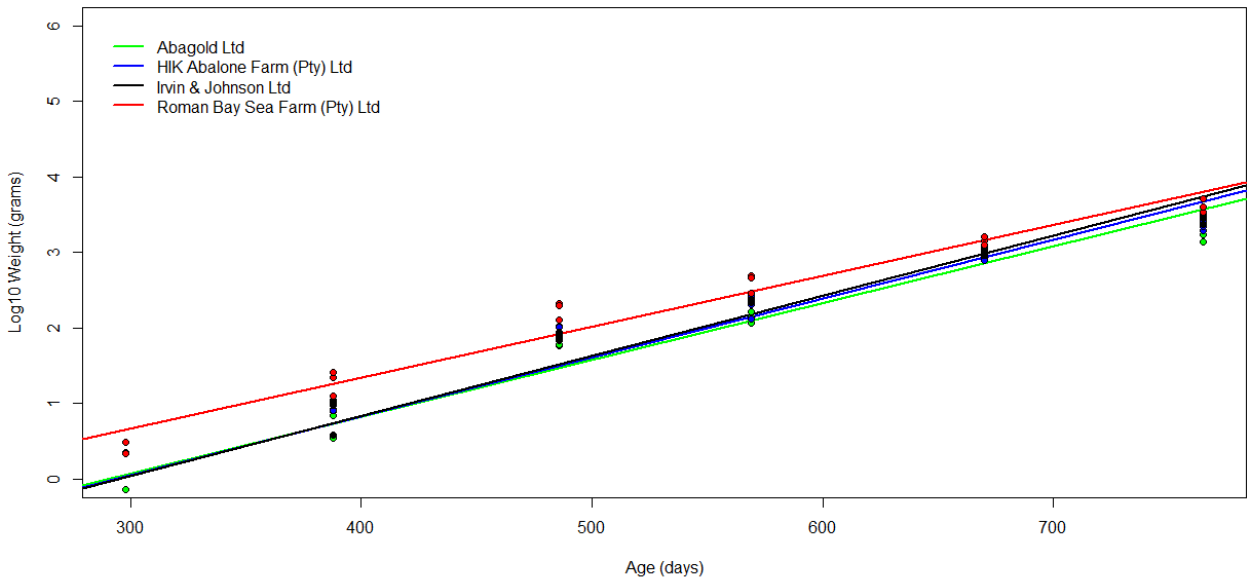

Figure 4.12 Weight-wise growth regression (g/d) of location HIK Abalone Farm (Pty) Ltd over four cohorts, over a period of 24 months.

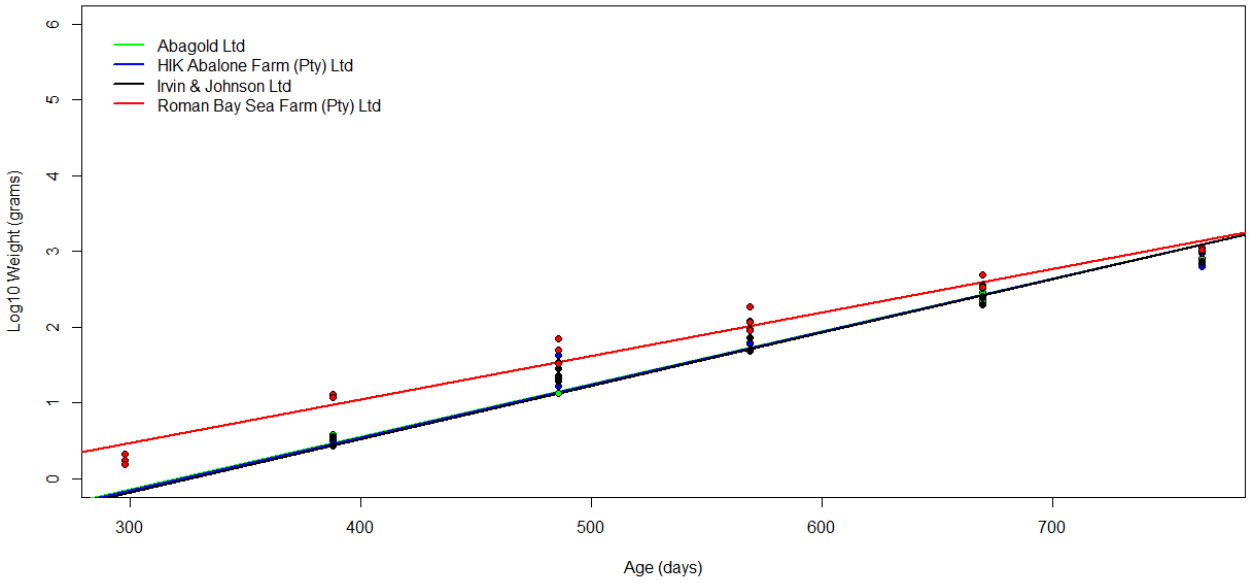

Figure 4.13 Weight-wise growth regression (g/d) of location Irvin & Johnson Ltd over four cohorts, over a period of 24 months.

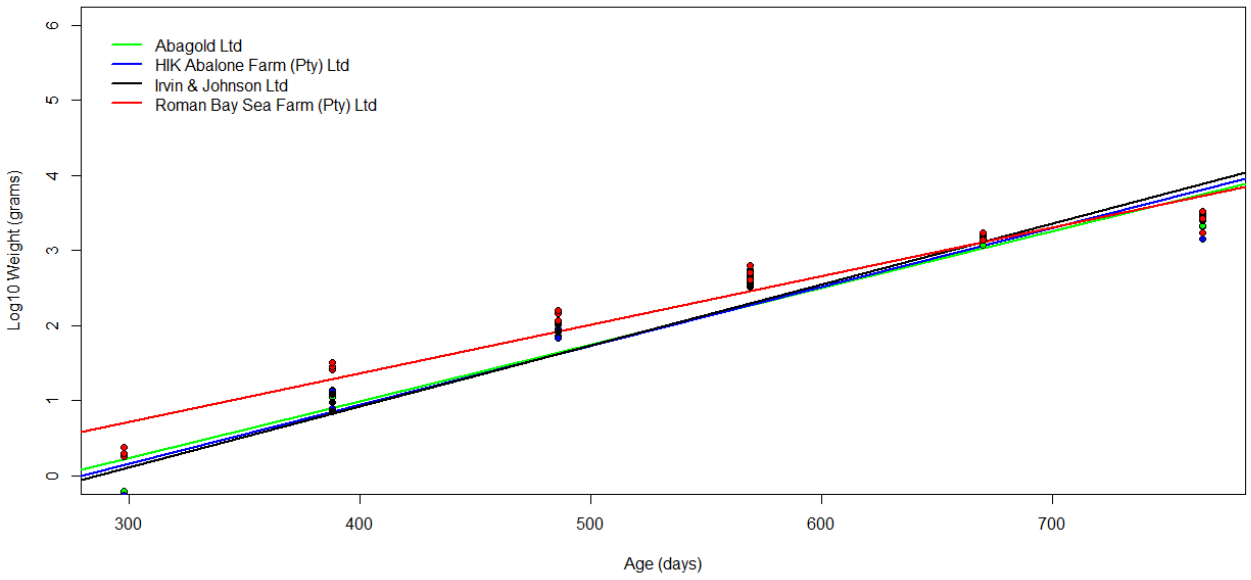

Figure 4.14 Weight-wise growth regression (g/d) of location Roman Bay Sea Farm (Pty) Ltd over four cohorts, over a period of 24 months.

4.3.5 Analysis of pairwise differences length gain regression (b_l) between locations.

4.3.5.1 Pairwise testing of statistically significant differences in length gain regression (b_l) between locations

The pairwise differences in log-transformed weight gain, b_w, between locations are displayed in Table 4.26 using multiple Bonferroni-adjusted t-tests

Table 4.26 Pairwise differences between locations in terms of lengthgain (b_l) (Bonferroni adjusted).

Least Squares Means for effect Location

i/j	Aqua	HIK	I&J	RB
Aqua		0.0001	<.0001	<.0001
HIK	0.0001		<.0001	1.0000
I&J	<.0001	<.0001		<.0001
RB	<.0001	1.0000	<.0001	

P<0.05 considered as significant

Table 4.27 A t-test of the average daily length gain (b_l) of the four locations.

Location	Mean b_l value	t-grouping	Graph
RB	0.08419	a	Figure 3.18
HIK	0.08349	a	Figure 3.16
Aqua	0.07690	b	Figure 3.15
I&J	0.06336	c	Figure 3.17

The graphs representing the performance of each cohort over all locations are presented in Figures 4.15 to 4.18. Each graph is a representation of a cohort's ADWG regressed over age over the four locations. The t-test values were converted into more interpretable and useful units and are presented in Table 4.28 below

Table 4.28 Useful growth parameters of locations based on average length gain (b_l).

Location	Growth per month (mm/m)	Days to 100mm
RB	2.5257	1187
HIK	2.5047	1198
Aqua	2.307	1300
I&J	1.9008	1578

4.3.5.2 Graphical representation of statistically significant differences in length gain regression (b_l) between locations

Figures 4.15 to 4.18 display the performance of the cohorts over the four locations as the regression of ADLG over age in days. The growth rate is expressed in units of mm per day

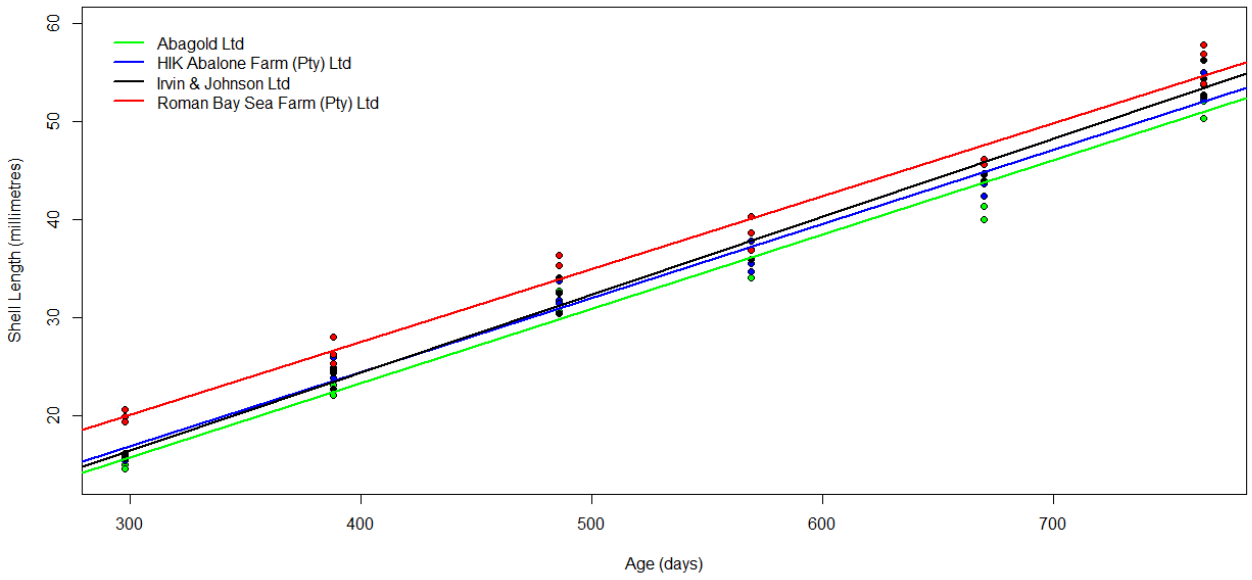

Figure 4.15 Length-wise growth regression (mm/d) of location Aquafarm Development (Pty) Ltd over four cohorts, over a period of 24 months.

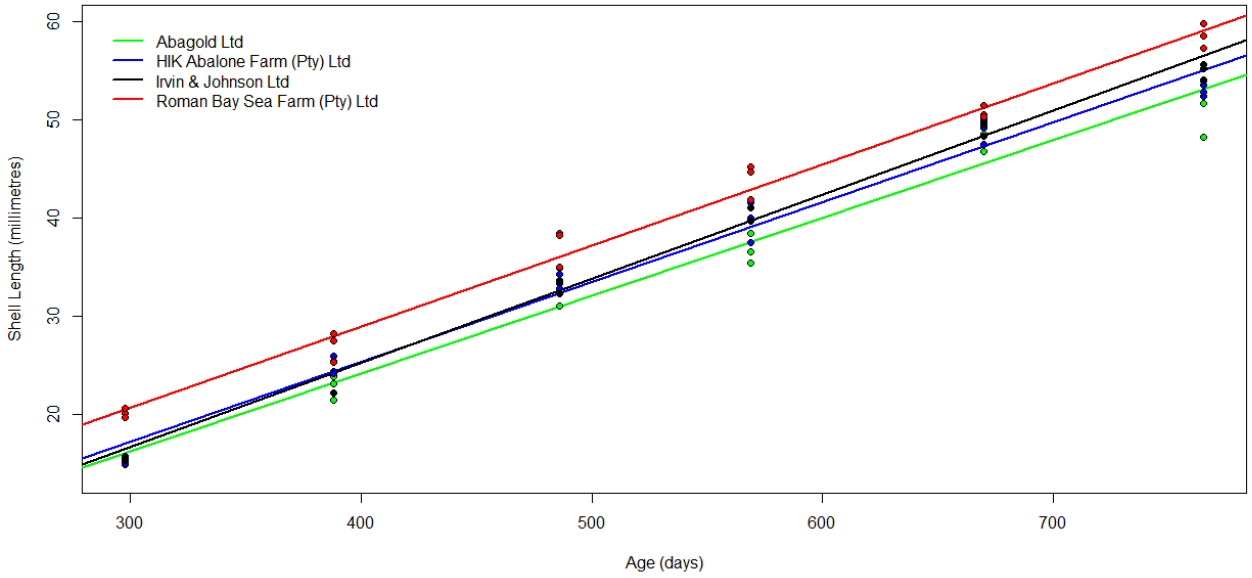

Figure 4.16 Length-wise growth regression (mm/d) of location HIK Abalone Farm (Pty) Ltd over four cohorts, over a period of 24 months.

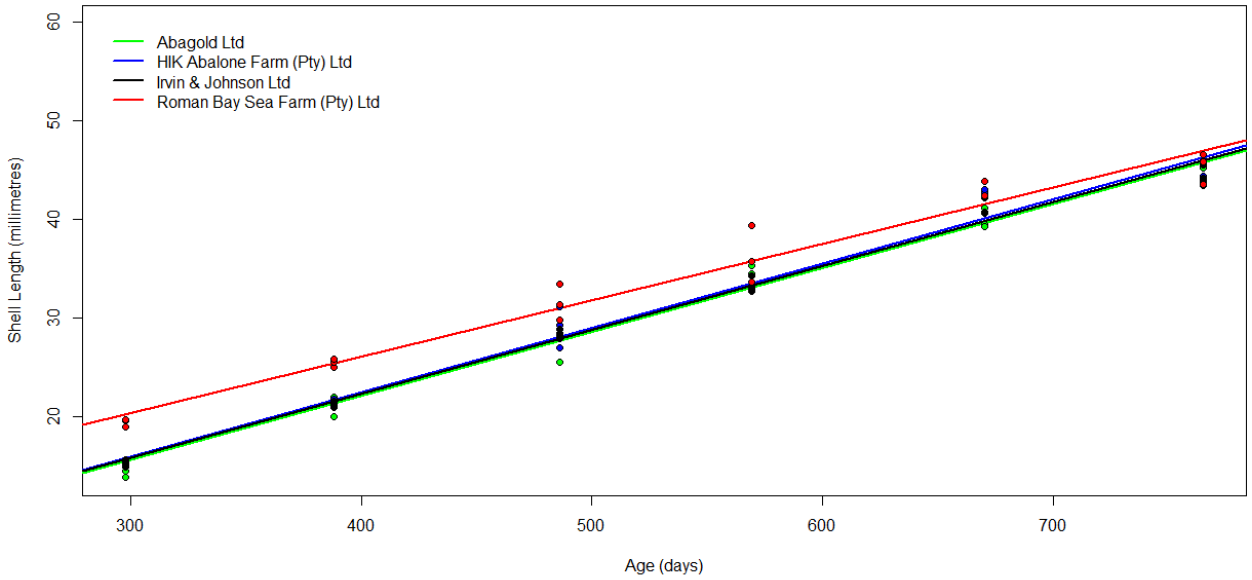

Figure 4.17 Length-wise growth regression (mm/d) of location Irvin & Johnson Ltd over four cohorts, over a period of 24 months.

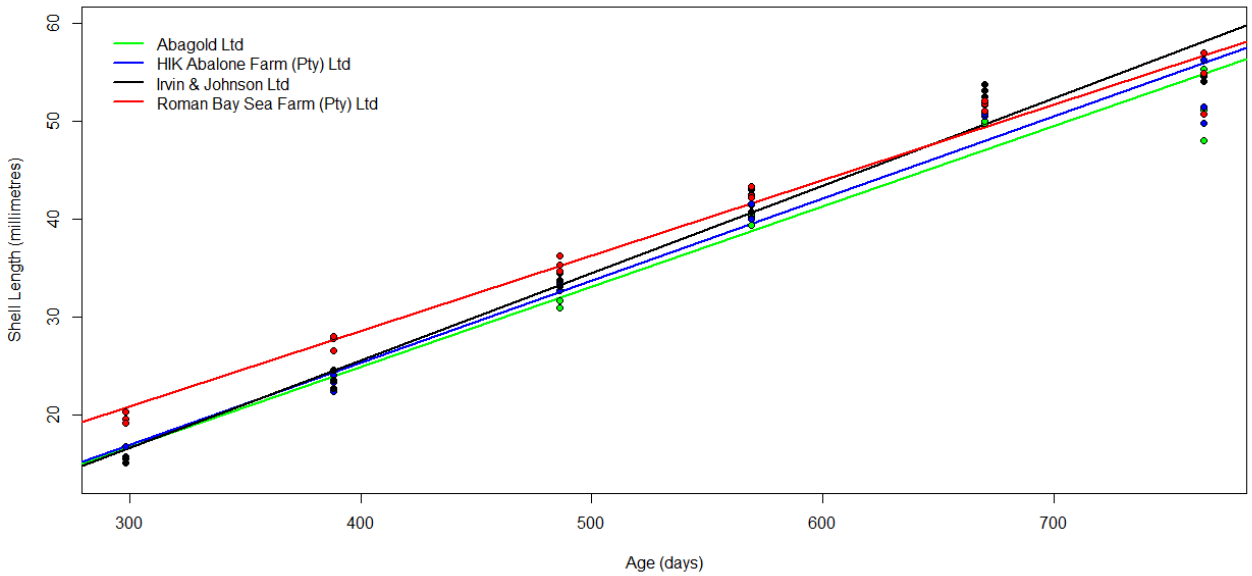

Figure 4.18 Length-wise growth regression (mm/d) of location Roman Bay Sea Farm (Pty) Ltd over four cohorts, over a period of 24 months.

4.4 Results and analysis of data corrected for differences in initial size of cohorts

The aim of the study was to compare the growth rates of the different cohorts and locations by assessing the regression coefficients of the growth rates over time. Some of the animals entered into the study and assigned to a cohort had a larger initial size at an equal age (Table 4.29). Larger abalone housed in the same basket are known to out compete smaller abalone for resources and it was suspected that this would affect the results of the study (Huchette *et al.*, 2003). A plot of initial weight and length against the growth rate displayed a moderate positive correlation (r=0.536 for length gain, and r=0.568 for weight gain) suggesting they share covariance.

Table 4.29 Initial size differences between cohorts at cohort assignment.

Cohort	Mean length (mm) and standard error	Mean weight (g) and standard error
Abagold Ltd	14.88±0.15	0.75±0.02
Aquafarm Development (Pty) Ltd	15.03±0.11	0.63±0.02
HIK Abalone Farm (Pty) Ltd	15.51±0.12	0.67±0.01
Irvin & Johnson Ltd	15.50±0.08	0.66±0.01
Roman Bay Sea Farm (Pty) Ltd	19.86±0.14	1.41±0.03

To correct for the initial size difference the initial mean length and initial mean weight from the first data recording were used as covariates. The results for the interaction model are presented in Tables 4.30 and 4.32 below. The analyses of variance for the main effect are presented in Tables 4.31 and 4.33.

Table 4.30 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for log-transformed weight gain (b_w) over a series of six measurements with initial weight entered as covariate.

Source	df	Mean square	F-ratio	P	adj-R^2
Initial Weight	1	2.316×10^{-6}	247.6466	2.480×10^{-16}	0.908
Cohort	3	1.5315×10^{-7}	16.3763	1.469×10^{-6}	
Location	3	5.2021×10^{-7}	55.6258	$1.397 \times 10^{-1}2$	
Cohort x Location	9	1.696×10^{-8}	1.8137	0.1055	
Error	31	9.35×10^{-9}			

Table 4.31 Analysis of variance table for main effects cohort and location on growth regression coefficients for log-transformed weight gain (b_w) over a series of six measurements with initial weight entered as covariate.

Source	df	Mean square	F-ratio	P	adj-R^2
Initial Weight	1	2.316×10^{-6}	209.322	2.2×10^{-16}	0.8912
Cohort	3	1.5315×10^{-7}	13.842	2.452×10^{-6}	
Location	3	5.2021×10^{-7}	47.017	3.506×10^{-13}	
Error	40	1.106×10^{-8}			

Table 4.32 Analysis of variance table for interaction model, and for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements with initial length entered as covariate.

Source	df	Mean square	F-ratio	P	adj-R^2
Initial Weight	1	4.457×10^{-5}	4.35	0.04533	0.8834
Cohort	3	3.2271×10^{-4}	31.4942	1.544×10^{-9}	
Location	3	8.8688×10^{-4}	86.5527	3.704×10^{-15}	
Cohort x Location	9	1.562×10^{-5}	1.5247	0.18302	
Error	31	1.025×10^{-5}			

Table 4.33 Analysis of variance table for main effects cohort and location on growth regression coefficients for length gain (b_l) over a series of six measurements with initial weight entered as covariate.

Source	df	Mean square	F-ratio	P	adj-R^2
Initial Weight	1	4.4457×10^{-5}	3.8907	0.05549	0.8697
Cohort	3	3.2271×10^{-4}	28.1686	5.876×10^{-10}	
Location	3	8.8688×10^{-4}	77.4132	2.2×10^{-16}	
Error	40	1.146×10^{-5}			

The analysis of variance for the interaction models (Tables 4.30 and 4.32) displayed no significant interaction for both the log-transformed weight gain coefficient (P=0.1055) and the length gain coefficient (P=0.18302). The ANOVA of the main effects for log-transformed weight gain (b_w) (Table 4.31) revealed statistically significant differences between the four cohorts (range from $0.0029 \pm 6.7 \times 10^{-5}$ to $0.0036 \pm 7.2 \times 10^{-5}$ log(grams) per day; $P=2.452 \times 10^{-6}$) and the four locations (range from $0.0031 \pm 7.5 \times 10^{-5}$ to $0.0035 \pm 9.2 \times 10^{-5}$ log(grams) per day; $P=3.506 \times 10^{-13}$). The same results were evident in the ANOVA for main effects on the regression coefficient for length gain (b_l) (Table 4.33). The four cohorts (range from 0.0735±0.0030 to 0.0807±0.0029 millimetres per day; $P=5.876 \times 10^{-10}$) and locations (range from 0.063±0.0011 to 0.0841±0.0016 millimetres per day; $P=2.2 \times 10^{-16}$) both displayed significant differences in b_l (Table 4.33).

4.5 References

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Chapter 5

Discussion

Chapter Five: Discussion

5. Discussion of results and analyses

5.1 Discussion of full model

Initial analysis was done on the raw data to assess whether there is a difference between the five breeding populations or cohorts, between the five locations and whether there were any interaction between the cohorts and locations. Plotting of the uncorrected data suggested that length growth followed a linear trend (Figure 4.1) and weight growth non-linear (Figure 4.2). The weight growth plots were therefore fitted to a quadratic equation and log-transformed to fit a linear model of regression.

The assumptions of normality and homoscedasticity for the interpretations of ANOVAs were tested and met. The analysis of variance of the full model revealed interaction for weight gain (P=0.0036) and length gain (P=0.035) between cohort and location (Table 4.1). A progressive consideration of possible factors are investigated and presented in sections 4.2.2 to 4.2.4.

The interaction between the cohort and location is important to consider when the environmental rearing factors cannot be controlled, but less important when these factors can be partially controlled (Gjedrem, 2012). In the case of this study the rearing conditions on the five farms (locations) have become fairly standardised with only some degree of variation in the feeding and stocking regimes. There is no general conclusion with regards to the interactions between genotype and environment (G x E) in aquatic species (Gjedrem, 2012). Varying levels of G x E interaction have been reported in aquaculture species. Low and insignificant G x E interaction has been reported in Atlantic salmon (*Salmo salar*) (Gunnes and Gjedrem, 1981), Nile tilapia (*Oreochromis niloticus*) (Eknath *et al.,* 1991) and shrimp (*Penaeus vannamei*) (Fjalestad *et al.,* 1997). Significant G x E interaction was found in Rohu carp (*Labeo rohita*) (Gjerde *et al.,* 2003). This inconsistency makes it vitally important to investigate the interaction between the cohorts and the locations in this study, and to determine the possible factors contributing to it.

5.2 Discussion of model corrected for progressive tag loss

An important aspect of the experimental design was that the cohorts or offspring from the five different breeding populations can be distinguished, to allow for communal housing of cohorts. The animals were initially tagged by inserting and lodging triangular silicone tags through the breathing pore of the animal. Each cohort or farm was represented by a different colour. This method proved ineffective and all the identifiable animals were retagged after three measurement intervals by gluing a small circular bee-tag to the shell of the individual animals. The effectiveness of this method was assessed in a study by Difford (2013). His findings suggested a significant amount of tag loss, especially after 12 months (Table 4.3). Some of the factors causing tag loss include human counting error, dislodging of the tag during handling, mortality and failed tag adhesion. All these factors were observed to act randomly and through observation it is assumed that no bias was introduced since the error was consistent among all treatments. The growth regression analysis is based on the means of samples and the decrease in sample sizes therefore increased the standard error of mean estimates. It is advised that animals be retagged as regularly as possible to prevent significant tag loss. To investigate the effect of the tag loss on the results of this study the last two sets of data were removed from the analysis.

The analysis of variance table (Table 4.4) for growth coefficients of log-transformed weight gain (b_l) again indicated statistically significant interaction between cohort and location (P=0.0105). No interaction was observed (P=0.4501) between the main effects on the regression coefficients of length gain (b_w) as presented in Table 4.5. This allowed for further testing of the main factors. Statistically significant differences were observed in the growth rate coefficients of both cohort (P=0.0009) and location (P<0.001) as presented in Table 4.6.

The pairwise comparisons of the significant differences in b_l between cohorts and locations were assessed in Tables 4.7 and 4.10 respectively by means of Bonferroni-adjusted t-tests. The mean b_l-value is expressed in units of millimetre growth per day. This unit is hard to interpret and therefore the results were converted into units that are more manageable. The results of the conversions are presented in Tables 4.9 and 4.11. Comparison of the b_l-values indicates Irvin & Johnson Ltd as the leading cohort (b_l=0.07915) and Roman Bay Sea Farm (Pty) Ltd as the worst performing (b_l=0.07216) in terms of ADLG. The exact opposite is true for the comparison of

locations with Roman Bay Sea Farm (Pty) Ltd (b_l=0.08372) significantly outperforming all but one [HIK Abalone Farm (Pty) Ltd] of the locations and Irvin & Johnson Ltd having the lowest b_l (0.0630).

5.3 Discussion of model corrected for progressive tag loss and farm management error

The consistency of the yellow silicone used to tag the animals assigned to Aquafarm Development (Pty) Ltd was harder and less elastic than those used for the other cohorts. This led to even greater tag loss. After four sets of measurements almost no identifiable animals remained in relation to this cohort. The growth rate regression coefficients of the Aquafarm Development (Pty) Ltd cohort was therefore based on only four data sets and could affect the results and conclusions of the study. It was therefore decided to exclude Aquafarm Development (Pty) Ltd as a cohort from the study after four sets of measurements.

The baskets housing the animals entered into the study were marked properly as to not be subjected to on farm practices such as sorting according to size which introduces selection. A management error at Abagold Ltd led to the animals being sorted along with the commercial animals on the farm after the fifth set of measurements. Because of this Abagold Ltd was excluded from the study as a location after five sets of measurements.

The study used the regression of average daily length gain (ADLG) (b_l) and average daily weight gain (ADWG) (b_w) as an indication of comparative growth rates. Therefore Aquafarm Development (Pty) Ltd could still be used as cohort and Abagold Ltd as location even though they only contributed four and five sets of measurements respectively. To assess the effect these errors had on the analysis, Aquafarm Development (Pty) Ltd was removed as cohort and Abagold Ltd was removed as location.

The analysis of variance table (Table 4.13) for growth coefficients of log-transformed weight gain (b_l) for the corrected model showed no significant interaction between cohort and location (P=0.3789). This is a deviation from the previous model where Aquafarm Development (Pty) Ltd was still present as cohort and Abagold Ltd as location. This ANOVA suggests that the referred errors contributed to the observed interaction in the previous models. Statistically significant differences in log-transformed weight gain (b_w) were observed (Table 4.14) between cohorts ($P < 0.001$) and between locations ($P<0.001$). The analysis of variance table for the interaction model

for length gain (b_l) (Table 4.15) also showed no significant interaction between cohort and location. The ANOVA for the main effects for length gain (b_l) (Table 4.16) indicated significant differences for both cohorts ($P<0.001$) and locations ($P<0.001$).

Pairwise comparative testing for the differences between cohorts in terms of log-transformed weight gain (b_w) and length gain (b_l) are presented in Tables 4.17 and 4.20 respectively. As expected the t-test tables (Tables 4.18 and 4.21) shows the ranking order for cohorts remained the same for both $\log_{10}(b_w)$and b_l. Irvin & Johnson Ltd performed the best as cohort and Roman Bay Sea Farm (Pty) Ltd displayed the slowest average growth rate. The performance of each cohort over the four locations is presented in Figures 4.3 to 4.6 for $\log_{10}(b_w)$and Figures 4.7 to 4.10 for b_l.

The differences in cohort growth rates can be attributed to several factors. The most obvious being the difference in initial size of abalone from different cohorts. Animals interact and failing to account for socially influenced traits can lead to selection in the opposite direction as seen in livestock such as pigs (Camerlink *et al.*, 2014), chicken (Peeters, 2012) and quail (Muir, 2012); but more prominently in fish where growth dispensations are an issue (Khaw, 2015). In this study genotype replicates were randomly rotated between measurements so the same replicates were not sharing baskets, however within replicate growth dispensations still occur and these inflate variance and lower group means. In an expanded breeding programme these interactions may be something to be aware of. The size of the abalone at the age of cohort assignment and tagging are affected by environmental conditions such as the feeding and husbandry practices of the participating farms. Biological aspects in the larval stage such as the variation in survival and fecundity also impacts on the stocking density of animals prior to cohort assignment. Cohorts with lower stocking densities during this stage had more favourable conditions in terms of habitat and feed availability (Vorster, 2003). The age at which abalone are moved from settlement to weaning also affects their size at the age they are tagged. Different growth dispensations were observed within baskets due to larger animals out competing smaller abalone for resources (Huchette *et al.*, 2003). The environmental effects are therefore not only in the climatic sense, but also the competition and dominance within a basket or husbandry unit. To investigate the effect of initial size on the regression coefficient analysis the initial weight or length was entered into the model as a covariate. The ANOVAs revealed no interaction between the main effects for $\log_{10}(b_w)$ (Table 4.30) and for b_l (Table 4.32). Furthermore the main effects displayed statistically significant differences between cohorts and between locations for both $\log_{10}(b_w)$ (Table 4.31) and b_l (Table

4.33). The results did not alter with the introduction of the covariate. Comparison of ANOVAs for b_l with (Table 4.32) and without (Table 4.15) the introduction of the covariate to the model showed an increase in the residual error ($9.94x10^{-6}$ vs $1.025x10^{-5}$). Despite the covariate being significant the increase in residual error when introducing the covariate suggests that it can be excluded from the model.

5.4 Discussion of statistically significant differences in cohorts and locations

Statistically significant differences were observed between the five cohorts and between the five locations in terms of length and weight growth rates. This was contrary to what was expected. It was assumed that the genetic diversity among the cohort populations is even and that no significant genetic differentiation between populations exists.

A molecular study of the population structures used in this study was conducted by Swart (2012) and confirmed that the broodstock used to construct the populations were representative of wild populations as no genetic differentiation was detected between the broodstock and the wild populations they were sourced from. The genetic diversity levels in the offspring populations were largely similar to wild populations with only Abagold Ltd and Aquafarm Development (Pty) Ltd experiencing a loss of diversity from broodstock to offspring populations. This is explained by the fact that the parental contribution of broodstock to offspring is highly variable in broadcast spawning molluscs such as *Haliotis midae* (Slabbert *et al.*, 2009). This reduces the effective population size. Reproductive success rates as low as 40% have been reported in commercial abalone (Elliot, 2000). Several factors impact on the high variability of reproductive success rates including spawning, the synchronising of spawning in males and females (Grubert *et al.,* 2005) and post-larval survival rates (Takami *et al.,* 2002; Symonds *et al.,* 2012). The unequal contribution of broodstock to progeny groups is especially problematic in broadcast-spawners (Dominik *et al.,* 2013). Dominik *et al.* (2013) simulated a breeding programme in greenlip (*Haliotis laevigata*) abalone for 10 generations (35 years) to estimate genetic gains in inbreeding depression based on different levels of reproductive failure. The simulation illustrated that twice the genetic gains can be achieved when high levels of inbreeding are tolerated. The study concluded that the variation in reproductive success rates can be compensated for through strategic breeding. The variation of reproductive contribution in the broodstock affects the number of families available for selection in the next generation. If the number of families is reduced after a spawning event, more related

individuals will be selected in the next generation. The level of relatedness cannot be assumed (Dominik *et al.,* 2013). The family structures in this study were impossible to determine due to the nature of the broadcast-spawning event, and the differential contribution of the broodstock to the progeny groups or cohort populations. The error variance can be underestimated, especially in traits with high heritability and lead to a high frequency of "false" significant differences between populations (Ponzoni, 2013). It was concluded that the genetic diversity between the study populations are not equal due to differential parental contribution of the broodstock to progeny groups.

All the cultured F_1 populations used in this study displayed significant genetic differentiation from their respective broodstock populations on molecular level (Swart, 2012). Van der Merwe (2009) investigated the genetic structure of nine wild populations of *H. midae* from different geographical regions along the South African coastline. The study found moderate to high levels of genetic variation exists among the nine populations. F_{IS} and F_{ST} values obtained from microsatellite marker analyses of these populations revealed genetic differentiation within populations was greater than between populations. Further analysis using single nucleotide polymorphisms corroborated these findings (van der Merwe, 2009).

Rhode (2013) conducted a study on wild and cultured populations of *H. midae* and found highly significant genotypic differences between wild and cultured populations and amongst cultured populations. This is in accordance to the findings of this study where statistically significant differences were found in phenotypic growth traits.

Another factor contributing to the difference observed in growth rates between cohorts is that there is very high variability during the stages prior to settlement, thus before tagging and cohort assignment (Kube *et al.*, 2007).

Several factors contributed to the significant differences in growth rates between locations. This includes managerial aspects such as stocking densities, food supply and water quality. Environmental influences also impact on growth rates. The ambient water temperature and water quality are the main contributing factors in this case (Vorster, 2003). The differences in between the average growth rates can be explained by exposure to the varying managerial and environmental factors between the five locations (Falconer, 1996). The ranking of locations is not

of interest to us in this study, but it shows that there are differences between the locations even though they are geographically very close. This provides useful information for developing a breeding programme as we can see comparing over locations adds noise to the analyses.

In conclusion, the aims of the study were met. The five cohorts and locations displayed statistically significant differences in terms of average length and weight growth.

5.5 Recommendations

This study revealed unexpected statistically significant differences between cohorts and between locations. Molecular studies that were conducted on the populations revealed the phenotypic differences in average growth rates can be partially attributed to differential parental contribution of broodstock. It is essential in future studies to incorporate molecular technologies to verify parentage and the contribution of the broodstock to the population. The high variability in growth during the stage prior to tagging and cohort assignment influences the initial size of the abalone entered into the study. The larger abalone hold a competitive advantage over smaller abalone when competing for habitat and food (Huchette *et al.*, 2003). This effect was compensated for in the analysis by entering the initial size as a covariate, however it is advised that the rearing stages prior to settlement be standardised. Since there are so many factors influencing these stages, a central facility should be used to effectively compare growth rates between different cohorts or populations. Failing these, repeating cohorts over locations to enable estimation and correction of location effects should multiple locations continue to be utilised for testing. The difference in management practices between locations after settlement may also impact on the observed differential growth rates further advocating the use of a central facility.

The single most limiting factor impacting on this study was the tagging of abalone and the subsequent progressive tag loss. If a better tagging method cannot be found it is advised that the identifiable animals be retagged at every measurement interval or at least every six months to ensure adequate sample sizes for a longer period of measurements. Better tagging methods would enable testing to the largest sales size and improve statistical power. Unreliable tagging is an issue which makes best linear unbiased prediction (BLUP) models for predicting breeding values and selection difficult. The use of genomic selection would be of benefit as a pedigree is no longer necessary, thus a reliable tagging method is not needed. An added bonus would be the reduced

generation interval as animals can be genotyped at weaning and be selected based on genomic predictions. Thousands of markers will however be needed.

The differences in means, residual error and number of replicates from this study can be used to estimate the number of replicates needed in future trials for genetic or nutrition purposes.

Any future research in selective breeding to follow this study should involve the integration of molecular techniques and biotechnologies. The integration of molecular population data with selective breeding programmes should be carefully considered. Reed and Frankham (2001) reported a weak correlation between molecular information and phonotypic performance. It is therefore suggested for a researcher to use molecular marker information at their own discretion when inferring a correlation between markers and the genetic performance of a strain (Ponzoni, 2013).

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